New Approach to Atherosclerosis from Studies of Chronic Granulomatous Disease

January 24, 2017
Demystifying Medicine Lecture

John I. Gallin, MD, Thomas Leto, PhD, Gal Wald, BA

NIH Clinical Center

Major Emphasis

- Study the pathophysiology of disease
- First in human with new therapeutics
- Study patients with rare diseases

18 – 25 million people in the United States have a rare disease!

Why Study Rare Diseases at the Clinical Center?

- Unusual ability to assemble cohorts of patients with rare diseases
- Provides hope to patients and their families
- Often provides insights to understanding common diseases

Rare Diseases: Window on Nature?

Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path;
nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature, by the careful investigation of cases of rarer forms of disease.

~ William Harvey, Letter IX, to John Vlackveld, *24 Apr 1657*

Outline for Today's Talk

- Atherosclerosis and Chronic Granulomatous Disease
- NOX family of NADPH Oxidases
- Journey to discover NOX inhibitors

The Story of Chronic Granulomatous Disease

A Rare Disease of Phagocytes

American Journal of Physiology, 103:235-236

THE EXTRA RESPIRATION OF PHAGOCYTOSIS

C. W. BALDRIDGE AND R. W. GERARD

From the Department of Physiology, University of Chicago

Received for publication August 17, 1932

As other cells increase their energy turnover during activity, it might be anticipated that the respiration of leucocytes would increase during active phagocytic ingestion—unless the entire process is controlled by physical tensions.

Experiments were performed, using Warburg manometers under conditions described elsewhere (Gerard and McIntyre, 1932). Seven-tenths

TABLE 1

Summary of eight pairs of experiments

TIME IN MINUTES	OXYGEN CONSUMPTION OF LEUCOCYTES, IN PER CENT INITIAL VALUE	
60	100	100
After tipping	Saline	Sarcina in saline
15	97	198
55	91	130
85	94	104
145	75	80

TABLE 2

April 14, 1932. Two-tenths cubic centimeter dog leucocyte paste in 0.6 cc. dog serum; 0.1 cc. N/10 NaOH in inset; onset, 0.2 cc. Ringer, plus $\frac{1}{10}$ ink or sarcina suspension.

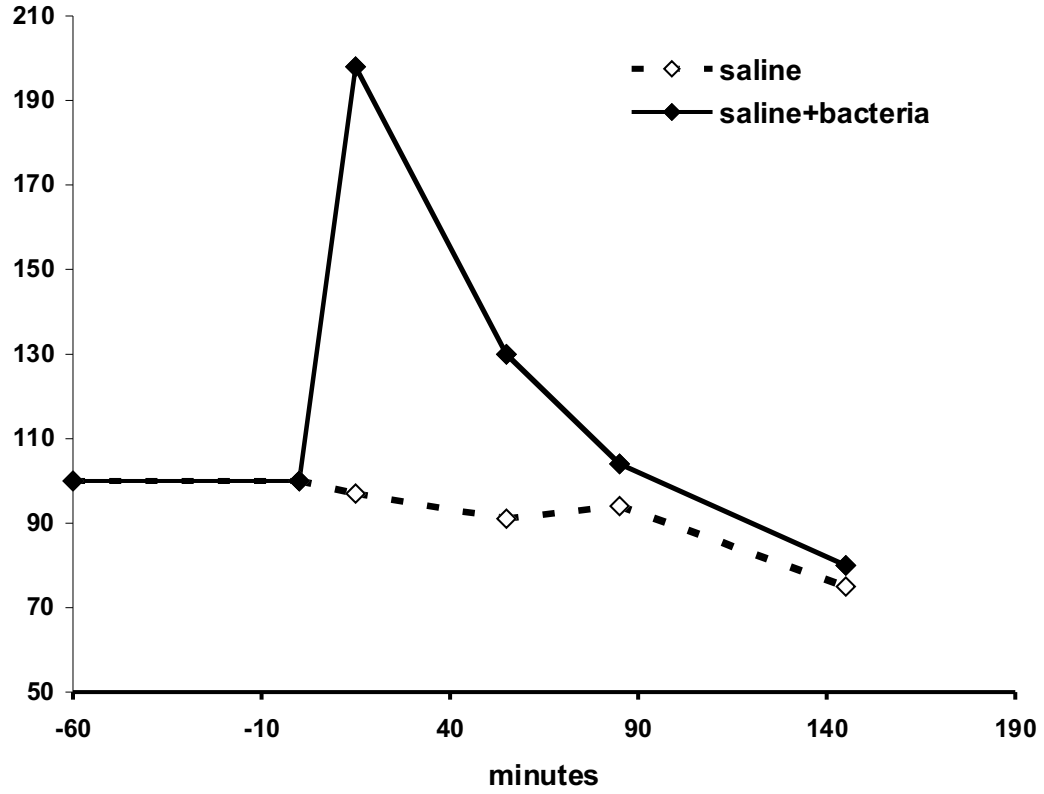
TIME IN MINUTES	OXYGEN CONSUMPTION OF LEUCOCYTES, IN PER CENT INITIAL VALUE		
70	100	100	100
After tipping	India ink	Sarcina in saline	Saline
15	93	423	154
45	95	128	104
105	100	81	88
165	75	78	98

Eight pairs of experiments showed a consistent increase in oxygen consumption after tipping the cocci, variable changes in the Ringer controls. The rise began at once, reached a maximum value twice that at the start in about 15 minutes, and was over in 90 to 150 minutes. Smears showed that the leucocytes were well loaded with organisms. The data are summarized in table 1 and a single protocol is reproduced in table 2.

It will be noted that the main burst of extra respiration lasts for 10 to 15 minutes, the maintained level being relatively little above the control, in individual cases below it. These may represent, respectively, the excess energy liberation of engulfment and digestion. Experiments with diluted india ink yielded negative results, the material appearing in several instances to be distinctly injurious.

BIBLIOGRAPHY

GERARD, R.W. AND M. MCINTYRE.
1933. This Journal, ciii, 225.



JAMA Pediatrics
Formerly Archives of Pediatrics & Adolescent Medicine
AMA Am J Dis Child. 1954;88:388-89

Society Transactions

AMERICAN PEDIATRIC SOCIETY

Rustin McIntosh, M.D., *President*

Sixty-Fourth Annual Meeting

Buck Hill Falls, Pa., May 3, 4, and 5, 1954

Hypergammaglobulinemia Associated with Severe Recurrent and Chronic Nonspecific Infection. DR. CHARLES A. JANEWAY, Boston, DR. JOHN CRAIG (by invitation), Boston, DR. MURRAY DAVIDSON (by invitation), New York, DR. WILLIAM DOWNEY (by invitation), New Bedford, Mass., DR. DAVID GITLIN (by invitation), Boston, and Julia C. Sullivan, M.P.H. (by invitation), Boston.

A Fatal Granulomatosus of Childhood:

The Clinical Study of a New Syndrome

HEINZ BERENDES, M.D.,
ROBERT A. BRIDGES, M.D.,
ROBERT A. GOOD, M.D.
Minneapolis, Minnesota

***Minnesota Medicine*, May 1957: 309-12**

In Vitro Bactericidal Capacity of Human Polymorphonuclear Leukocytes: Diminished Activity in Chronic Granulomatous Disease of Childhood

P. G. QUIE, J. G. WHITE, B. HOLMES, AND R. A. GOOD
(From the Departments of Pediatrics and Microbiology, University of Minnesota Medical School, Minneapolis, Minn.)

Journal of Clinical Investigation, Vol. 46, No. 4, 1967

The Journal of Clinical Investigation
Vol. 46, No. 9, 1967

Studies of the Metabolic Activity of Leukocytes from Patients with a Genetic Abnormality of Phagocytic Function *

BEULAH HOLMES,‡ ARTHUR R. PAGE,§ AND ROBERT A. GOOD ||

(*From the Pediatrics Research Laboratories of the Variety Club Heart Hospital and the Departments of Pediatrics and Microbiology, University of Minnesota, Minneapolis, Minnesota*)

Science
1967; 155: 835-6

Leukocyte Oxidase: Defective Activity in Chronic Granulomatous Disease

ROBERT L. BAEHNER
DAVID G. NATHAN
Department of Medicine, Children's Hospital Medical Center, and Harvard Medical School, Boston, Massachusetts

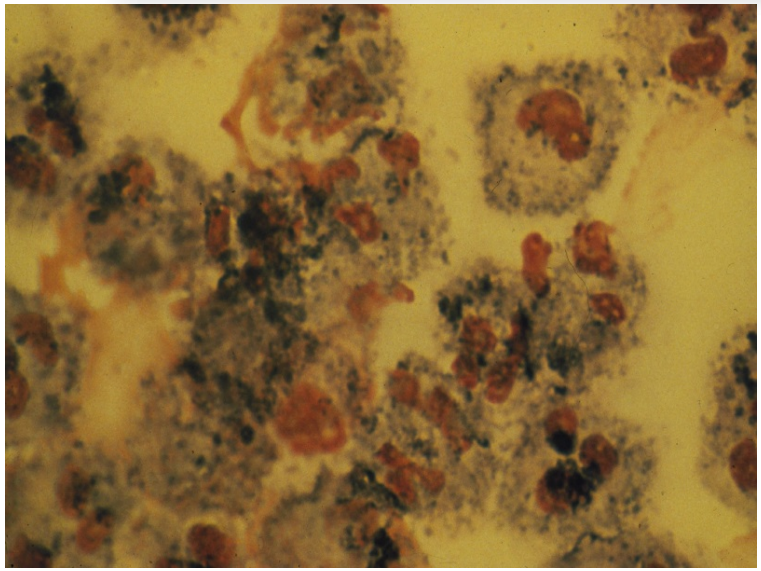
The New England
Journal of Medicine

Volume 278 MAY 2, 1968 Number 18

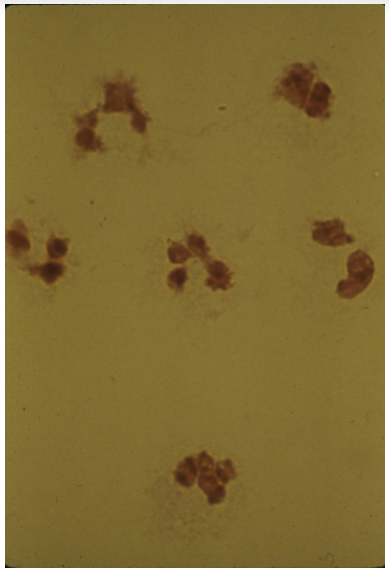
QUANTITATIVE NITROBLUE TETRAZOLIUM TEST IN CHRONIC GRANULOMATOUS DISEASE*

ROBERT L. BAEHNER, M.D., AND DAVID G. NATHAN, M.D.

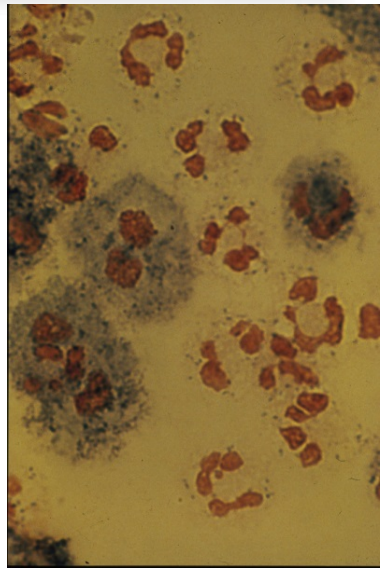
Department of Medicine, Children's Hospital Medical Center, and Harvard Medical School, Boston, MA



Normal



CGD

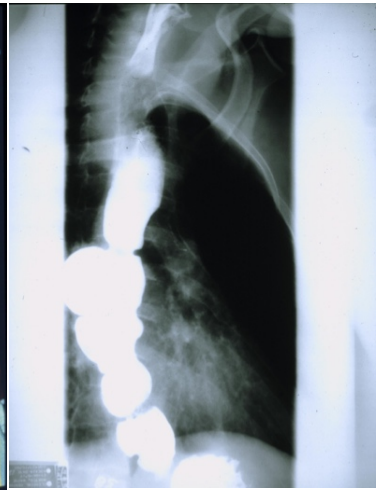


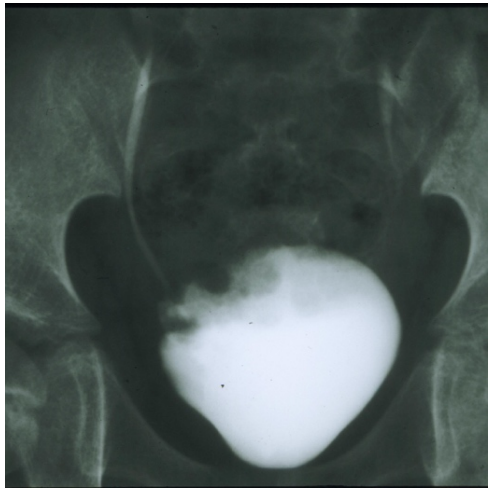
X-Linked

Chronic Granulomatous Disease (CGD)

- ~1:200,000 live births in US
- Mortality 2%/yr
- Abnormal NADPH Oxidase in phagocytes

Chronic Granulomatous Disease

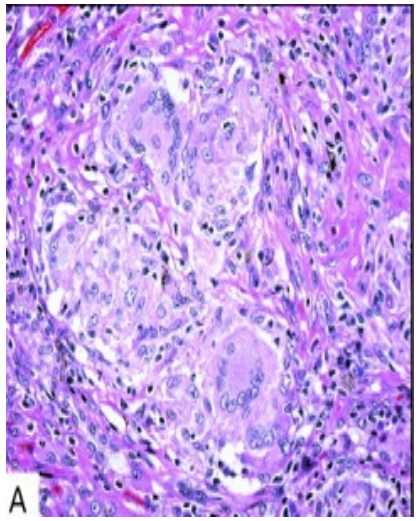




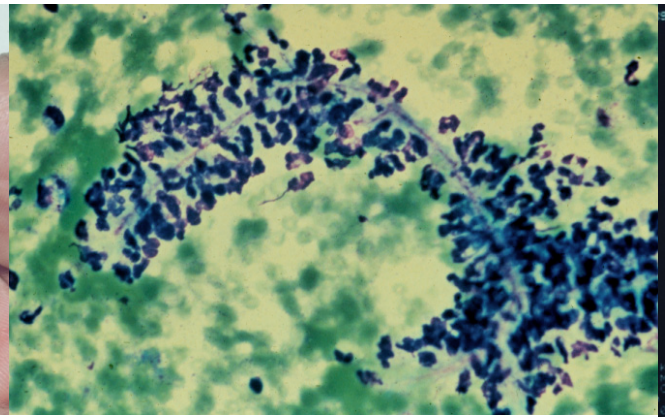
NADPH Oxidase

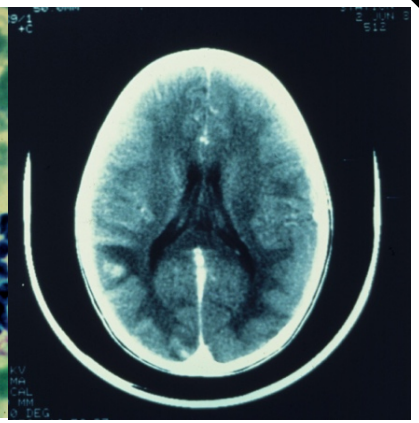
X

Granulomas



Infections





Nature
Vol. 32, No. 6074, 1986

Cloning the gene for an inherited human disorder—chronic granulomatous disease—on the basis of its chromosomal location

Brigitte Royer-Pokora*, Louis M. Kunkel†, Anthony P. Monaco†, Sabra C. Goff*, Peter E. Newburger‡, Robert L. Baehner*, F. Sessions Cole§, John T. Curnutte‖ & Stuart H. Orkin*¶#

* Division of Hematology-Oncology, The Children's Hospital, Dana-Farber Cancer Institute, Department of Pediatrics, † Division of Genetics, The Children's Hospital, Department of Pediatrics and the Program in Neuroscience, § Division of Cell Biology, The Children's Hospital, Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115, USA
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‖ Division of Pediatric Hematology, Department of Pediatrics, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA
¶ Howard Hughes Medical Institute, Children's Hospital, Boston, Massachusetts 02115, USA

Science
1989; 245: 409-12

Recombinant 47-Kilodalton Cytosol Factor Restores NADPH Oxidase in Chronic Granulomatous Disease

KAREN J. LOMAX,* THOMAS L. LETO, HIROYUKI NUNOI, JOHN I. GALLIN, HARRY L. MALECH

The Journal of Clinical Investigation
Vol. 86, No. 5, 1990

Human Neutrophil Cytochrome *b* Light Chain (p22-*phox*)

Gene Structure, Chromosomal Location, and Mutations in Cytochrome-negative Autosomal Recessive Chronic Granulomatous Disease

Mary C. Dinauer,* Eric A. Pierce,* Gail A. P. Bruns,‡ John T. Curnutte,§ and Stuart H. Orkin*‖

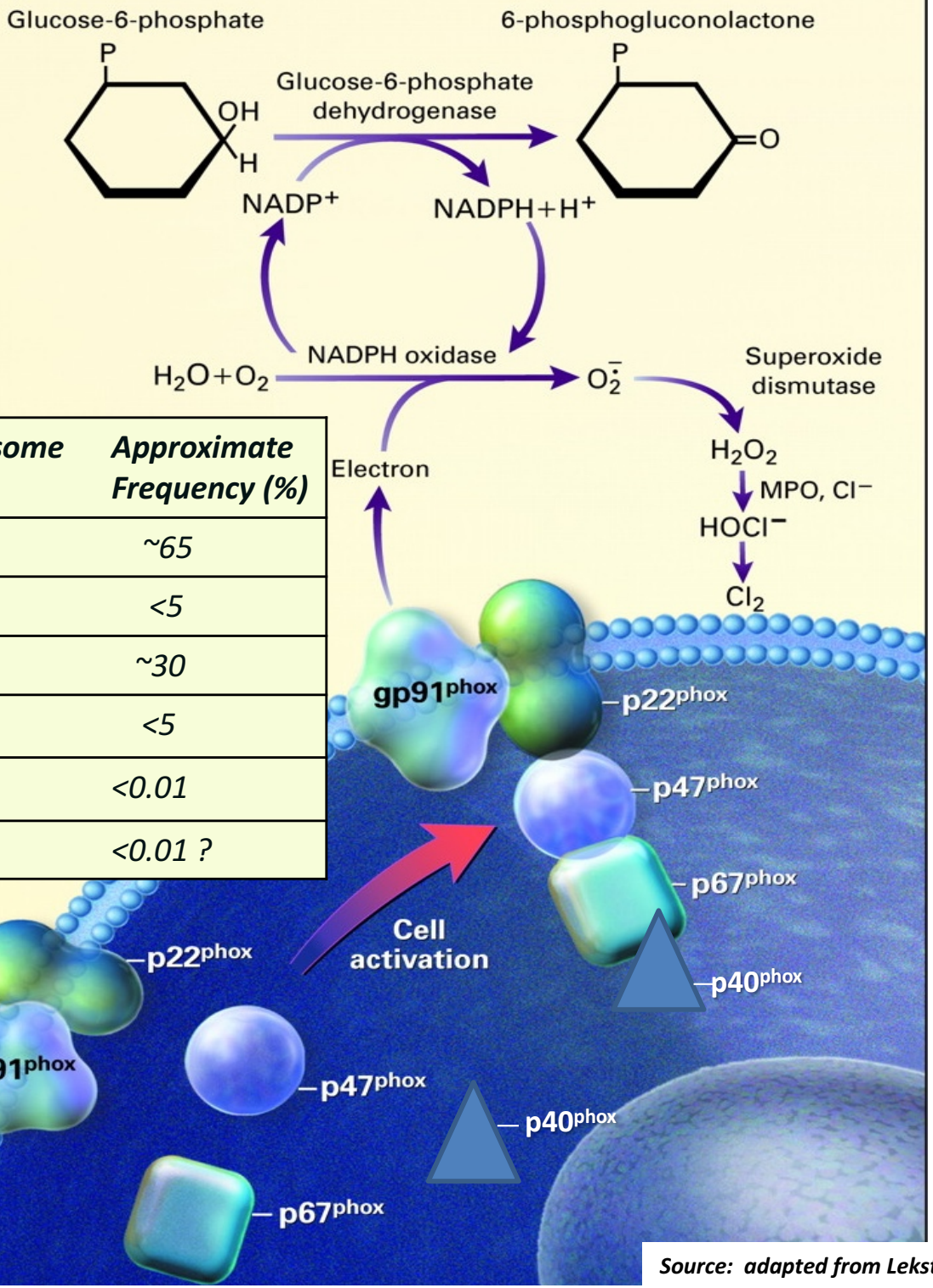
*Division of Hematology-Oncology, Children's Hospital, and the Dana Farber Cancer Institute, Department of Pediatrics, and ‡Division of Genetics, Children's Hospital, Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115; §Department of Molecular and Experimental Medicine, Scripps Clinic, La Jolla, California 92037; and ‖Howard Hughes Medical Institute, Boston, Massachusetts 02115

Science
1990; 248:727-730

Cloning of a 67-kD Neutrophil Oxidase Factor with Similarity to a Noncatalytic Region of p60$^{c\text{-}src}$

THOMAS L. LETO,* KAREN J. LOMAX, BRYAN D. VOLPP, HIROYUKI NUNOI, JOAN M. G. SECHLER, WILLIAM M. NAUSEEF, ROBERT A. CLARK, JOHN I. GALLIN, HARRY L. MALECH

Molecular basis for Chronic Granulomatous Disease (CGD)



Gene	*Chromosome*	*Approximate Frequency (%)*
gp91 phox	*Xp21.1*	*~65*
p22 phox	*16q24*	*<5*
p47 phox	*7q11.23*	*~30*
p67 phox	*1q25*	*<5*
G6PD	*Xq28*	*<0.01*
p40 phox	*22q13.1*	*<0.01 ?*

Source: adapted from Lekstrom-Himes, and Gallin,. NEJM; 343:23, 2000.

NIAID CGD Patients Followed at the NIH

CGD Genotype	Families	Patients	Carriers
gp91^{phox}	273	305	203
p47^{phox}	97	123	33
p67^{phox}	18	17	10
p22^{phox}	11	11	2
p40^{phox}	2	2	3
G6PD	1	1	1
Totals	367	419	214

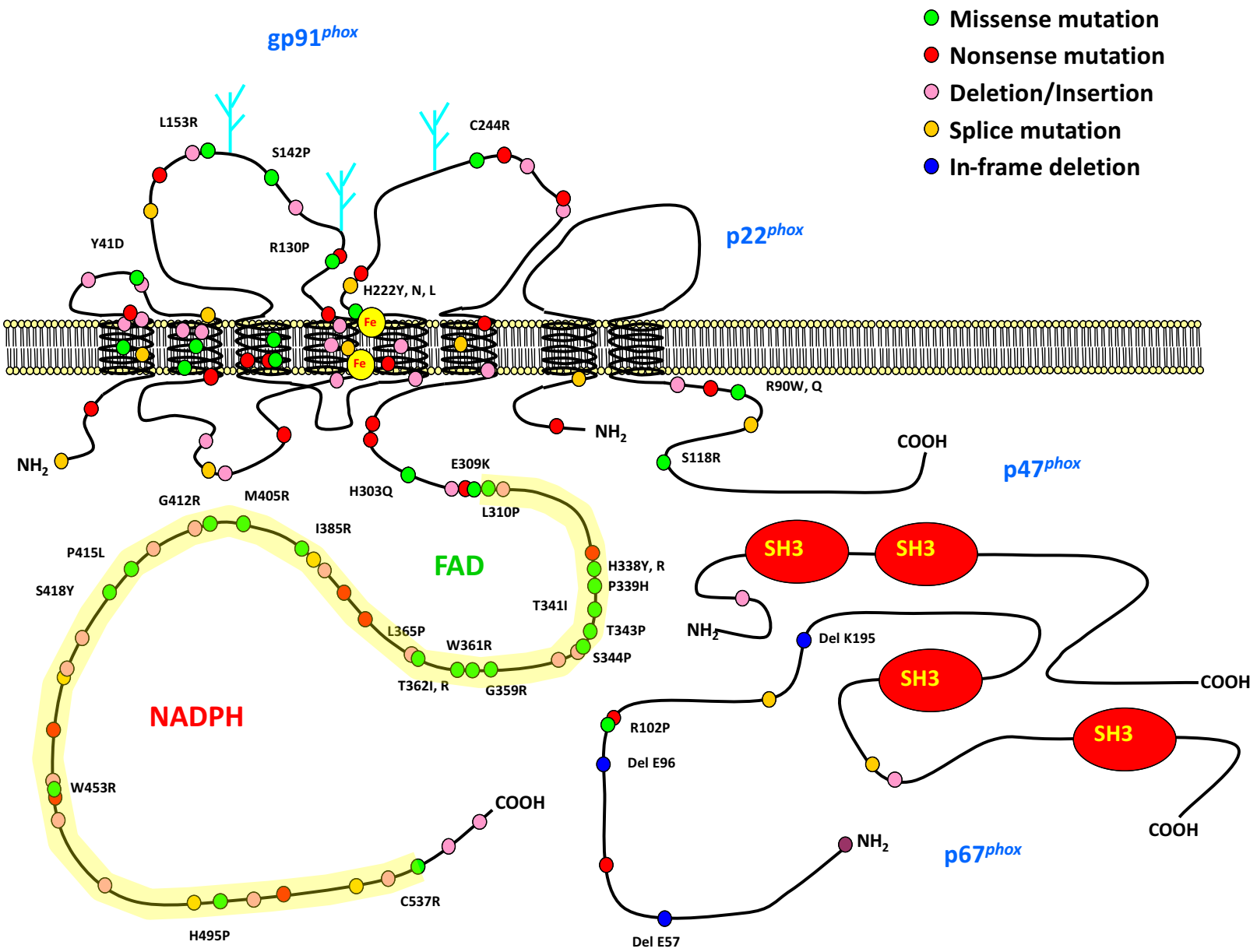
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

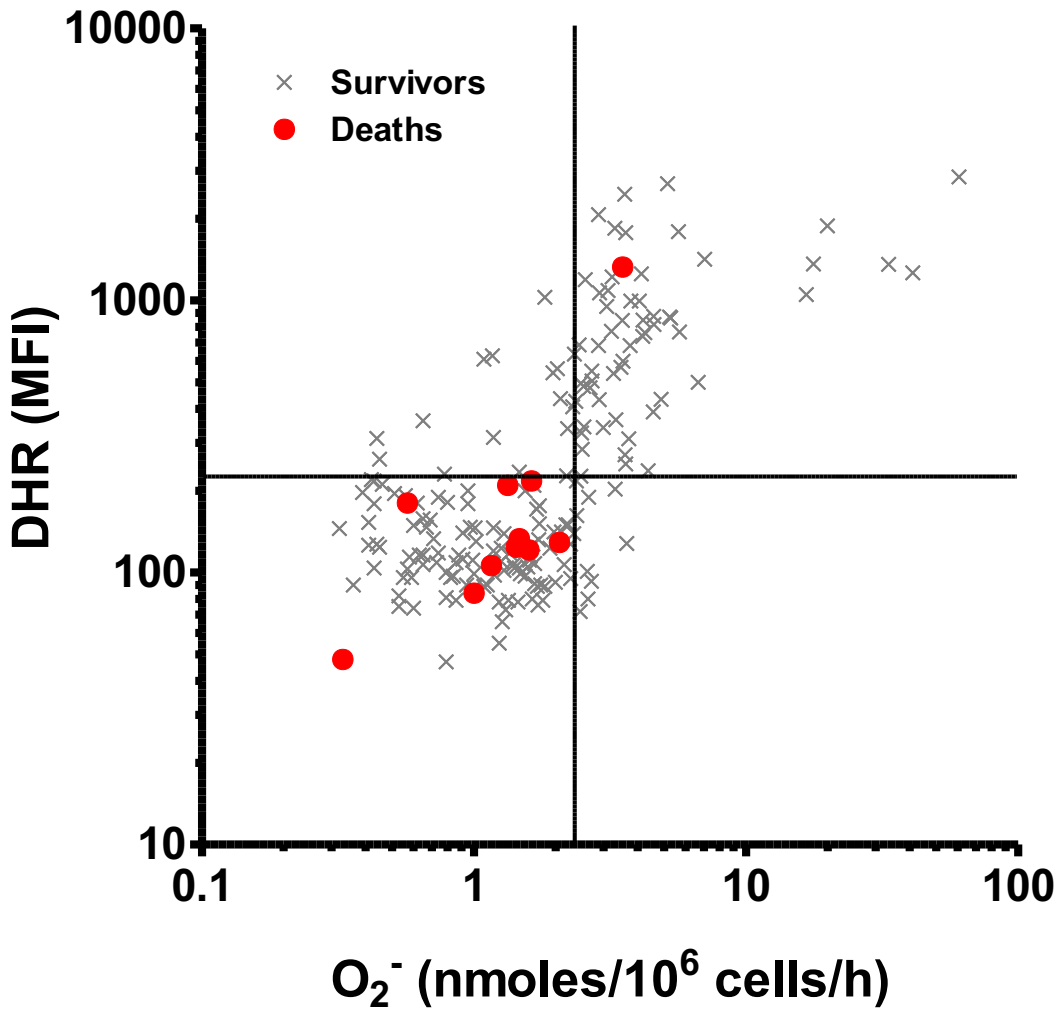
Residual NADPH Oxidase and Survival in Chronic Granulomatous Disease

Douglas B. Kuhns, Ph.D., W. Gregory Alvord, Ph.D., Theo Heller, M.B., Ch.B., Jordan J. Feld, M.D., M.P.H., Kristen M. Pike, M.S., Beatriz E. Marciano, M.D., Gulbu Uzel, M.D., Suk See DeRavin, M.D., Ph.D., Debra A. Long Priel, M.S., Benjamin P. Soule, M.D., Kol A. Zarember, Ph.D., Harry L. Malech, M.D., Steven M. Holland, M.D., and John I. Gallin, M.D.

N ENGL J MED 363;27 NEJM.ORG DECEMBER 30, 2010



Kuhns, et.al. *NEJM* 363: 2600-2610, 2010



Kuhns, et.al. *NEJM* 363: 2600-2610, 2010

Impact of the Study of Residual NADPH Oxidase in CGD

- **Enabled stratification of CGD patients based on residual respiratory burst activity and risk of mortality.**
- **Based on this study high risk patients seen with low or absent residual phagocyte ROS are now considered for bone marrow transplantation very early in life.**

Early Observations Suggesting Loss of NADPH Oxidase Function is Protective for Atherosclerosis

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Autopsies

Mouse studies

Violi, et.al.

p47*phox* is required for atherosclerotic lesion progression in *ApoE*$^{-/-}$ mice

Barry-Lane, P. et al, *J. Clin. Invest.* 108: 1513-1521, 2001

Vascular Medicine

Hereditary Deficiency of gp91^{phox} Is Associated With Enhanced Arterial Dilatation

Results of a Multicenter Study

Francesco Violi, MD; Valerio Sanguigni, MD; Roberto Carnevale, PhD; Alessandro Plebani, MD; Paolo Rossi, MD; Andrea Finocchi, MD; Claudio Pignata, MD; Domenico De Mattia, MD; Baldassarre Martire, MD; Maria Cristina Pietrogrande, MD; Silvana Martino, MD; Eleonora Gambineri, MD; Anna Rosa Soresina, MD; Pasquale Pignatelli, MD; Francesco Martino, MD; Stefania Basili, MD; Lorenzo Loffredo, MD

***Circulation*. 2009; 120: 1616 - 1622**

ARTICLE

Oxidative Stress Is Associated With Arterial Dysfunction and Enhanced Intima-Media Thickness in Children With Hypercholesterolemia: The Potential Role of Nicotinamide-Adenine Dinucleotide Phosphate Oxidase

Francesco Martino, MD[a], Lorenzo Loffredo, MD[b], Roberto Carnevale, PhD[b], Valerio Sanguigni, MD[c], Eliana Martino, MD[a], Elisa Catasca, MS[a], Cristina Zanoni, MS[a], Pasquale Pignatelli, MD[b], Francesco Violi, MD[b]

[a]Center of Clinic Lipid Research, Department of Pediatrics, and [b]Division of Internal Medicine H, Department of Experimental Medicine, University of Rome "La Sapienza," Rome, Italy; [c]Department of Internal Medicine, University of Rome "Tor Vergata," Rome, Italy

The authors have indicated they have no financial relationships relevant to this article to disclose.

***Pediatrics*. 2008; 122: e648 – e655**

Letter to the Editor

Nox2 Is Determinant for Ischemia-Induced Oxidative Stress and Arterial Vasodilatation: A Pilot Study in Patients With Hereditary Nox2 Deficiency

F. Violi
V. Sanguigni
L. Loffredo
R. Carnevale
B. Buchetti
A. Finocchi
M. Tesauro
P. Rossi
P. Pignatelli

Department of Experimental Medicine and Pathology (F.V., L.L., R.C., B.B., P.P.), University of Rome "La Sapienza", and the Departments of Internal Medicine (V.S., M.T.) and Experimental Medicine (A.F., P.R.), University of Rome "Tor Vergata," Rome, Italy.

***Arterioscler Thromb Vasc Biol*. 2006; 26: e131 – e132**

Clinical Protocol

NIH Protocol 10-I-0029 – Non-invasive Assessment of Atherosclerosis in Patients with CGD and Other Disorders of the Immune System-J Gallin, PI

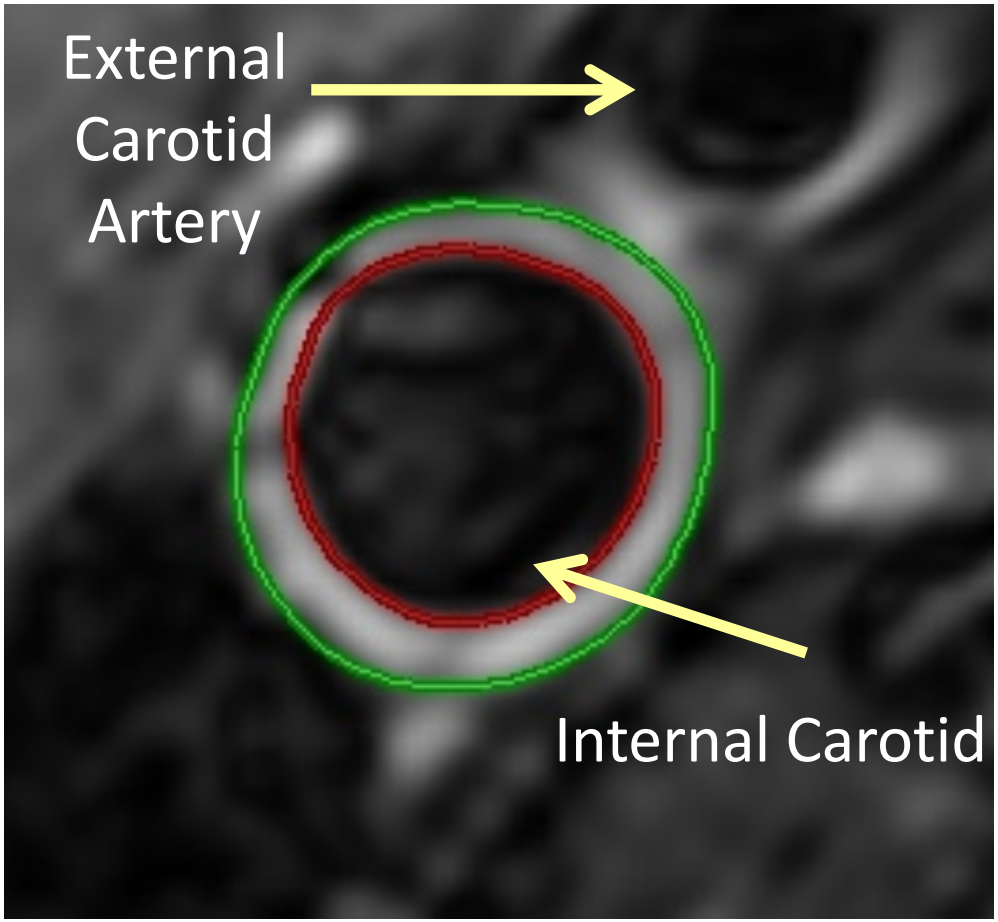
Question: Does loss of NADPH oxidase function in CGD result in decreased atherosclerosis?

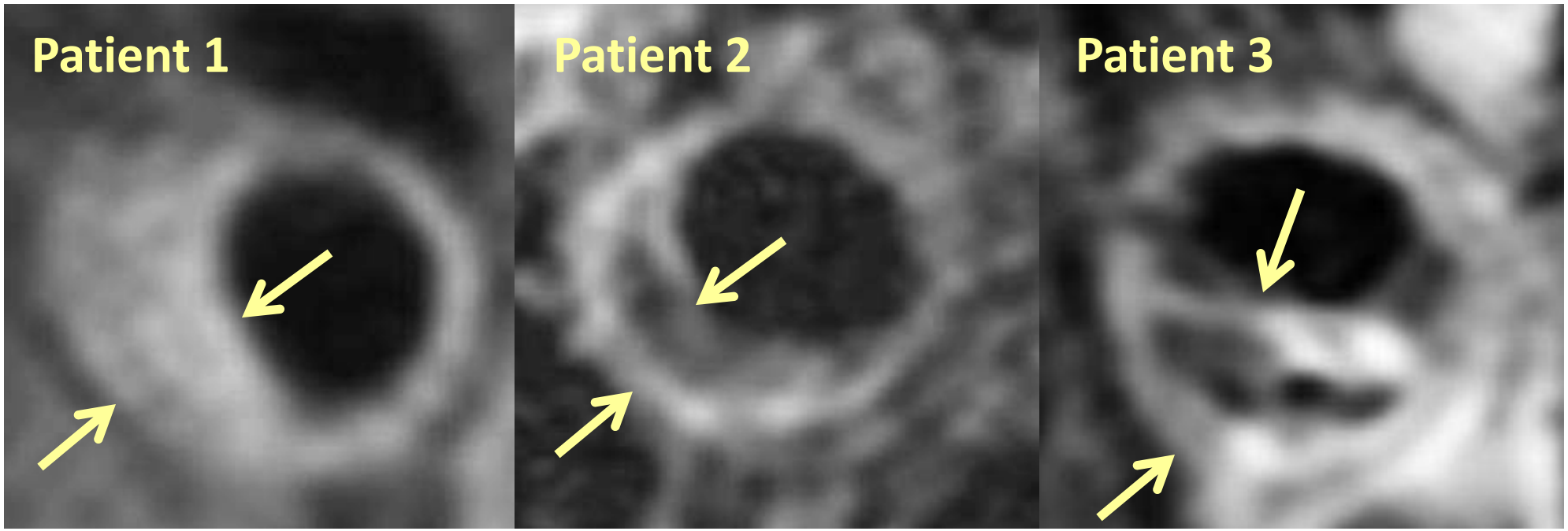
Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
130:2031-2039. 2014

American Heart Association®

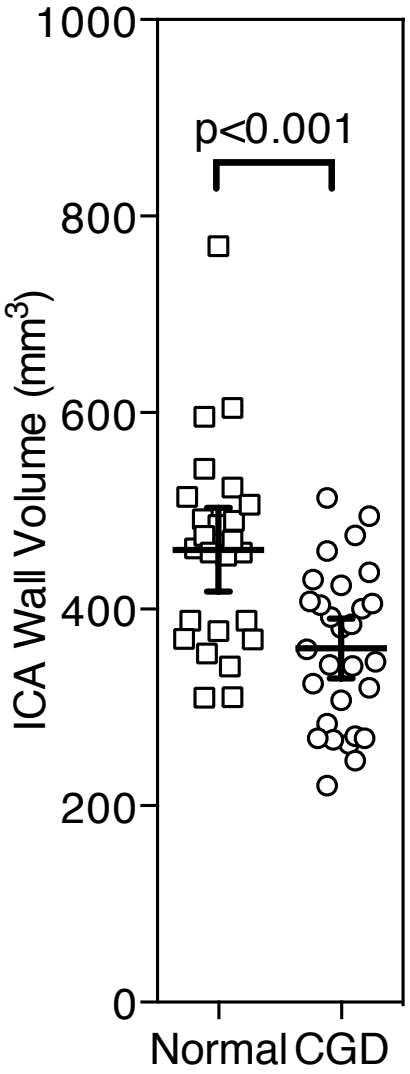
Assessment of Atherosclerosis in Chronic Granulomatous Disease

Christopher T. Sibley, Tyra Estwick, Anna Zavodni, Chiung-Yu Huang, Alan C. Kwan, Benjamin P. Soule, Debra A. Long Priel, Alan T. Remaley, Amanda K. Rudman Spergel, Evrim B. Turkbey, Douglas B. Kuhns, Steven M. Holland, Harry L. Malech, Kol A. Zarember, David A. Bluemke and John I. Gallin

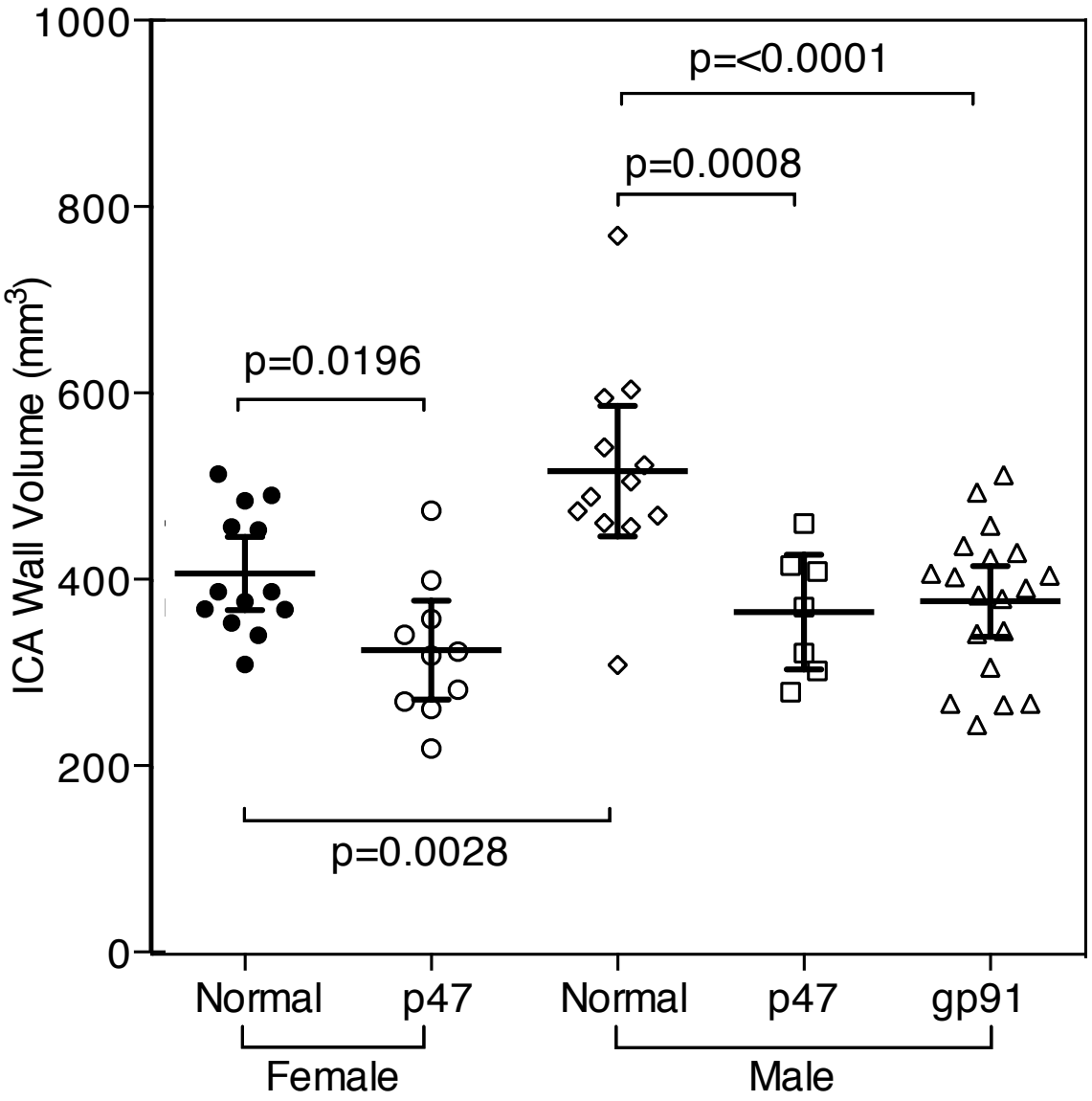

External
Carotid
Artery
Internal Carotid


Patient 1
Patient 2
Patient 3

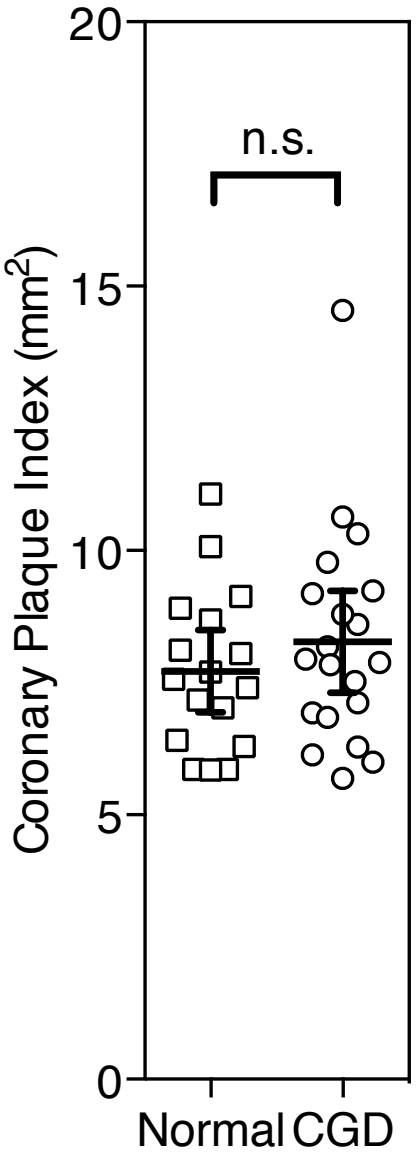
Internal Carotid Arteries of CGD patients have significantly smaller wall volumes than age-matched control subjects



ICA wall volume of both autosomal and X-linked CGD patients are significantly smaller than sex-matched healthy controls

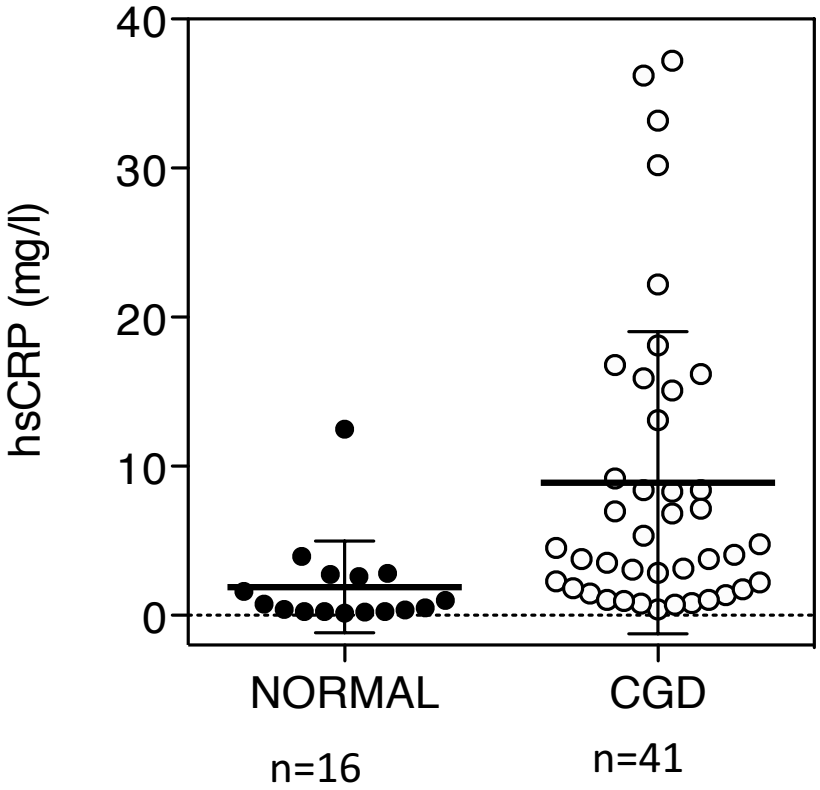


Coronary Plaque Index of CGD patients is similar to that of age-matched control subjects



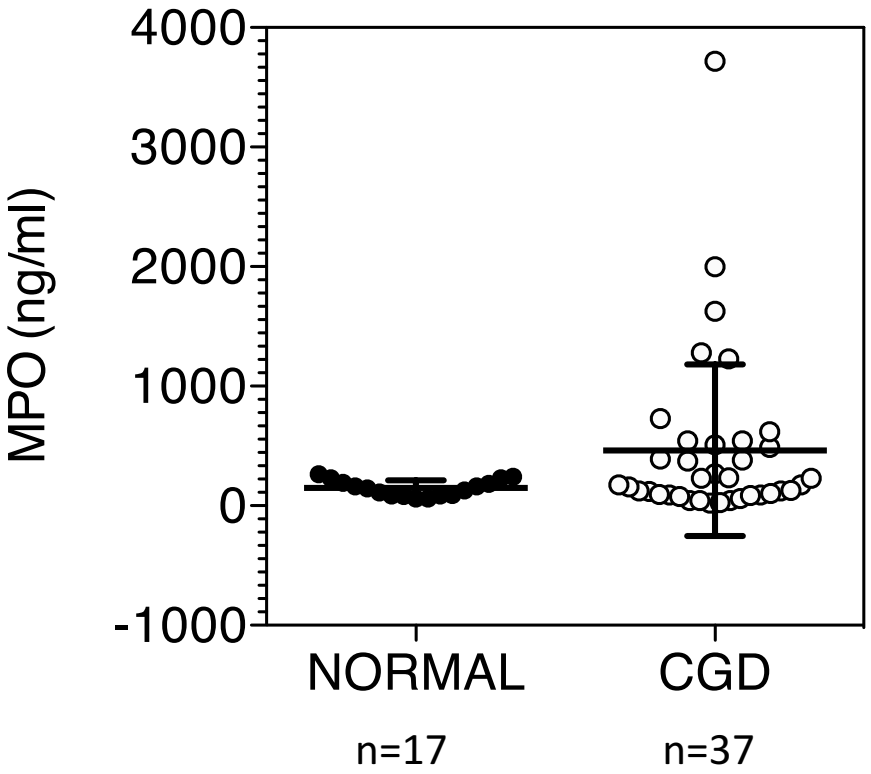
Risk Factors for Atherosclerosis

CRP



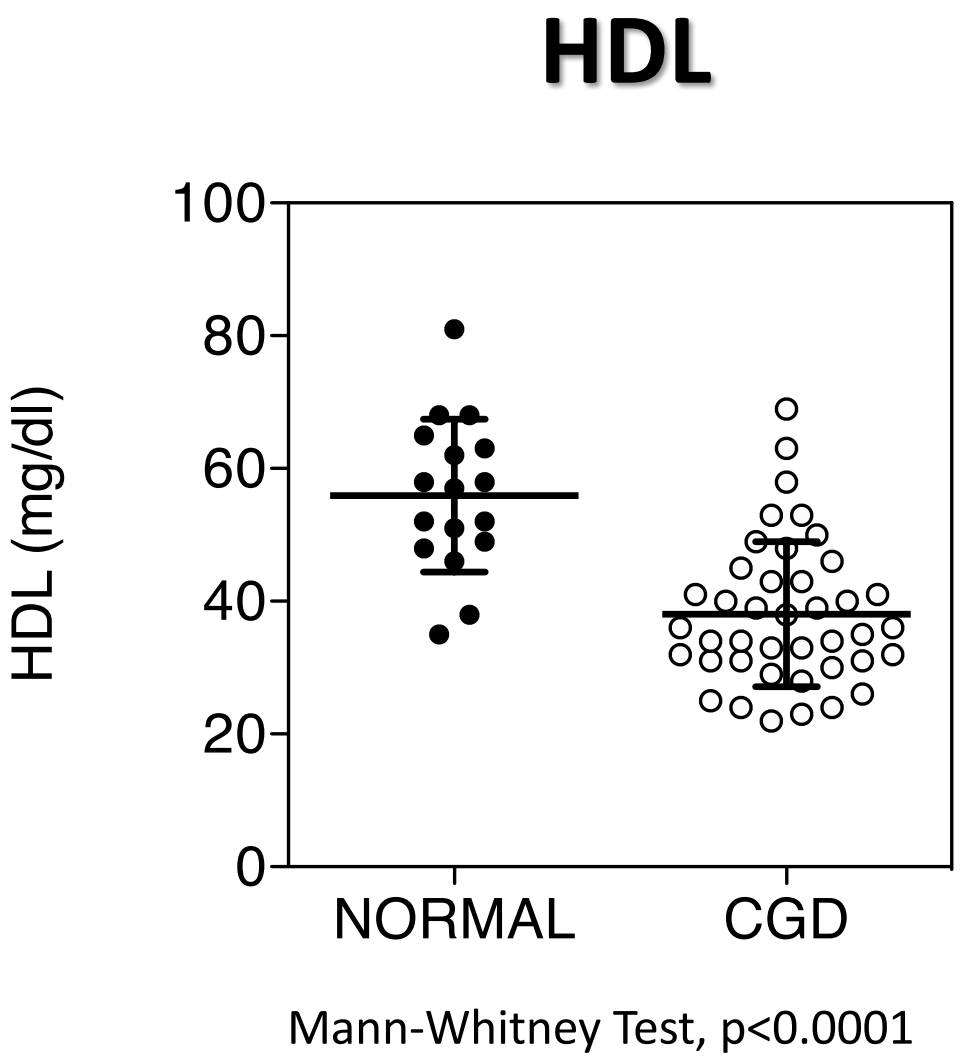
Mann-Whitney Test, p<0.0001

MPO



P = 0.0115 by unpaired t-test with Welch's correction

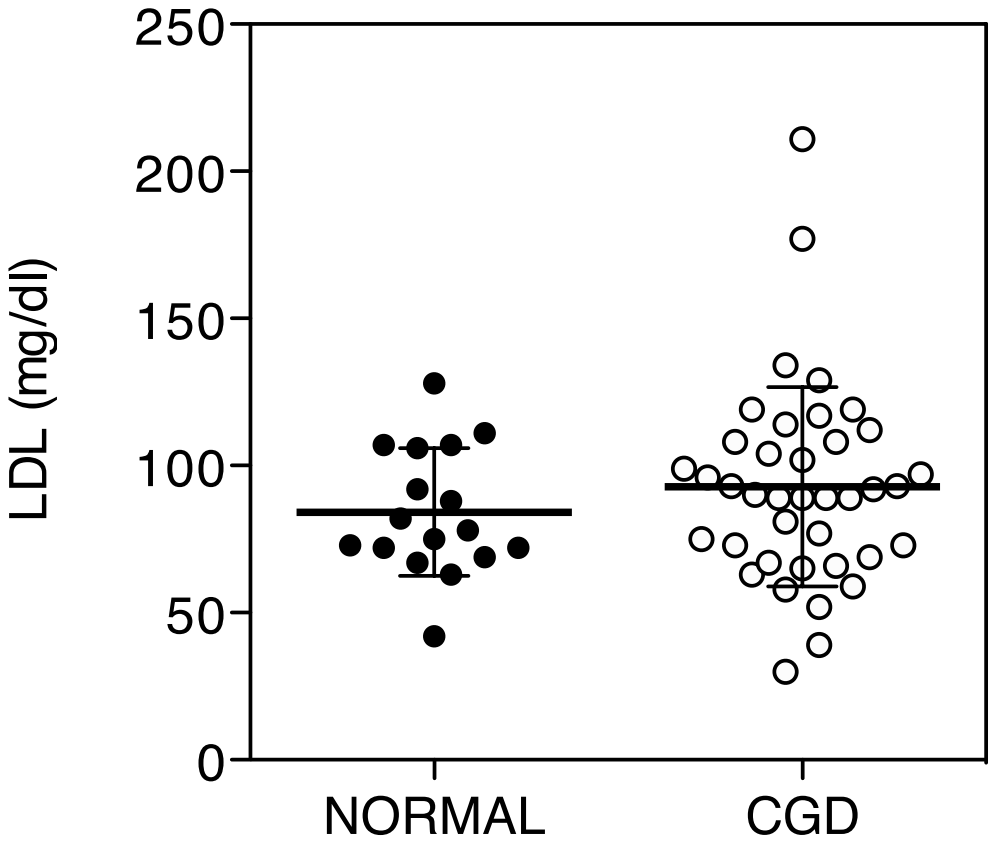
Risk Factors for Atherosclerosis



Mann-Whitney Test, $p<0.0001$

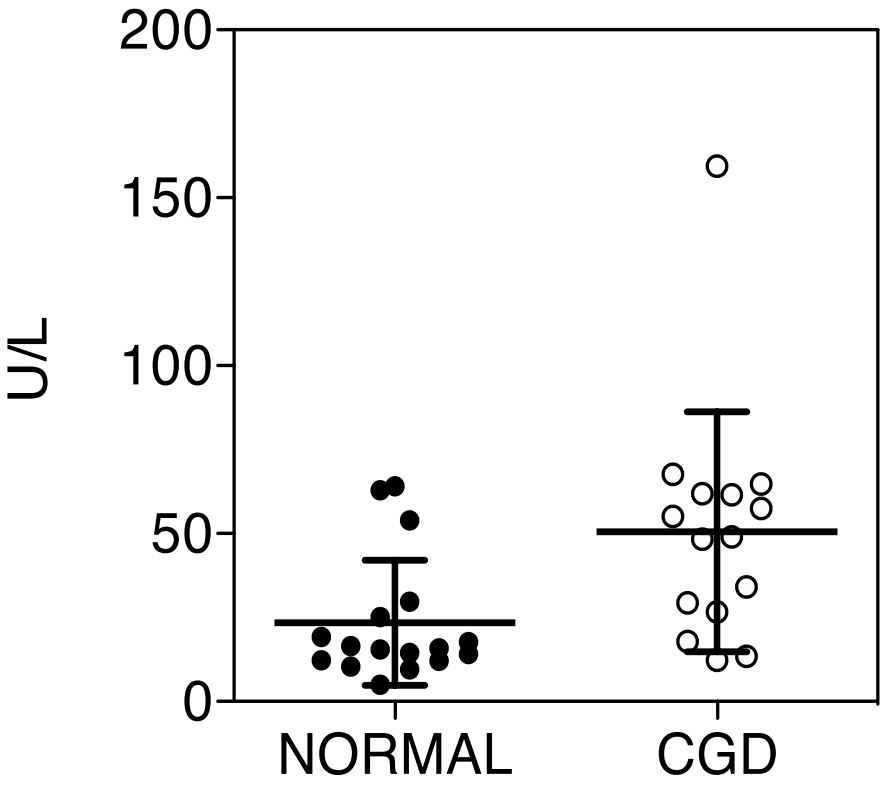
Risk Factors for Atherosclerosis

LDL



p=0.3873

Oxidized LDL



p=0.0073

Under review

Summary

1. **CGD patients are protected from carotid artery thickening despite adverse risk factors. This suggests absence of NOX 2 is protective of carotid artery atherosclerosis.**

2. **The increased risk factors of elevated MPO, CRP, oxidized LDL and low HDL in CGD delinks NOX 2 from these observations.**

Future Opportunity

Would ROS products missing in CGD be good therapeutic targets for preventing and possibly treating atherosclerosis and other inflammatory diseases?

NOX family NADPH Oxidases: Mediators of inflammation, fibrosis and cancer progression

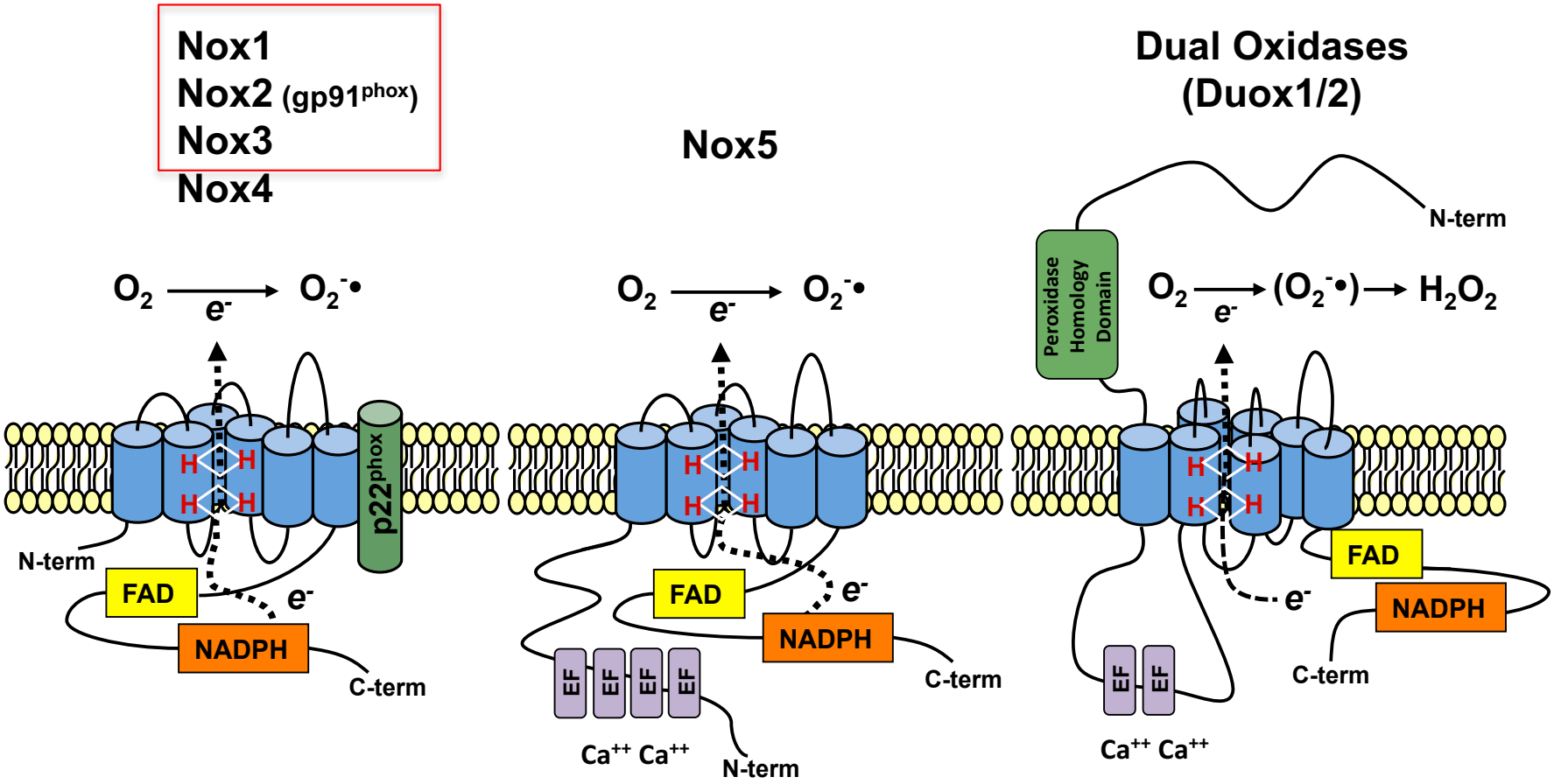
Thomas Leto, Ph.D.
National Institutes of Health (NIH)
National Institute of Allergy and Infectious Disease (NIAID),
Laboratory of Host Defenses (LHD)

NIH National Institutes of Health
Turning Discovery Into Health

NIAID

Nox family NADPH Oxidases:

Diverse ROS generators

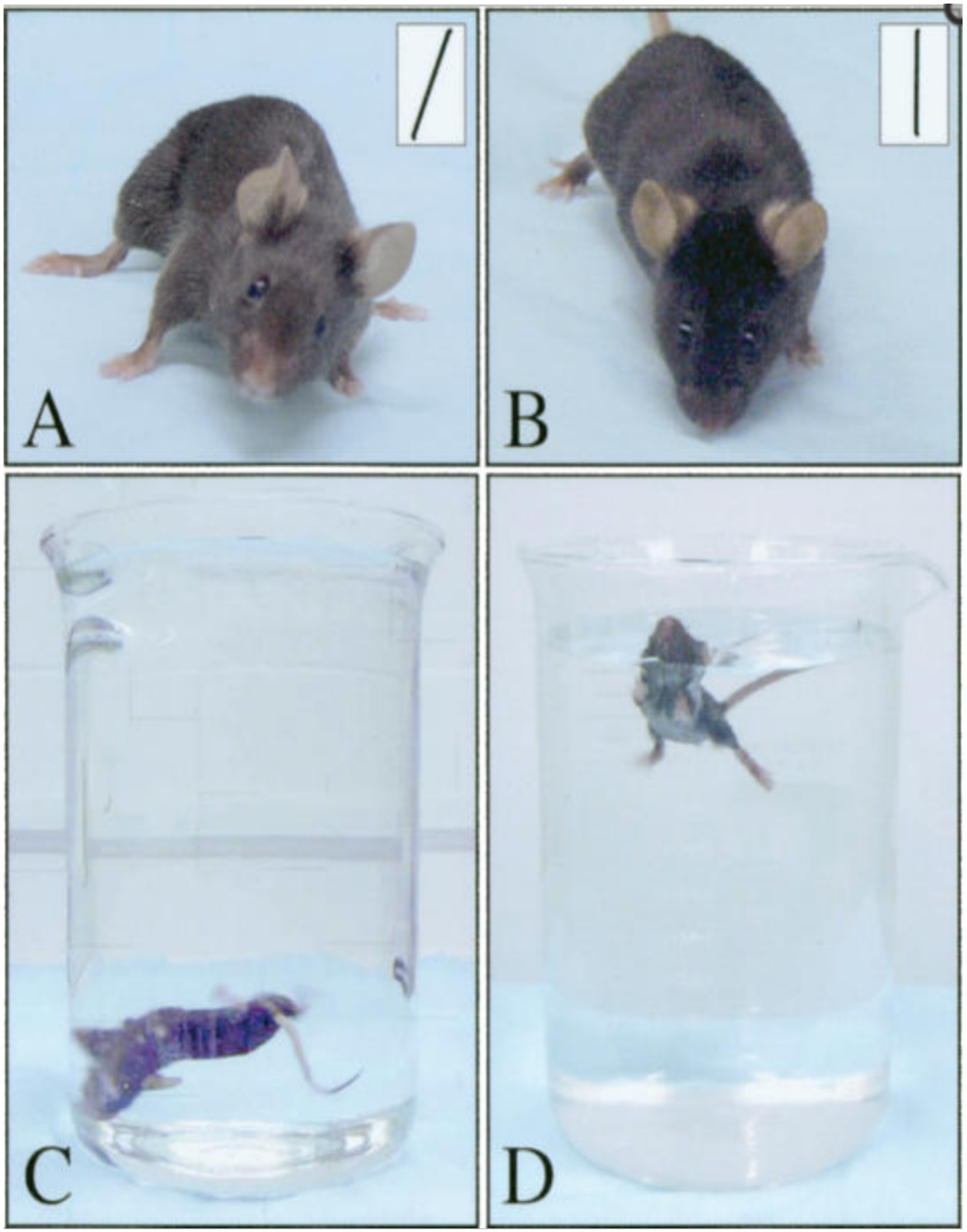


Disease phenotypes linked to Nox gene disruptions/mutations

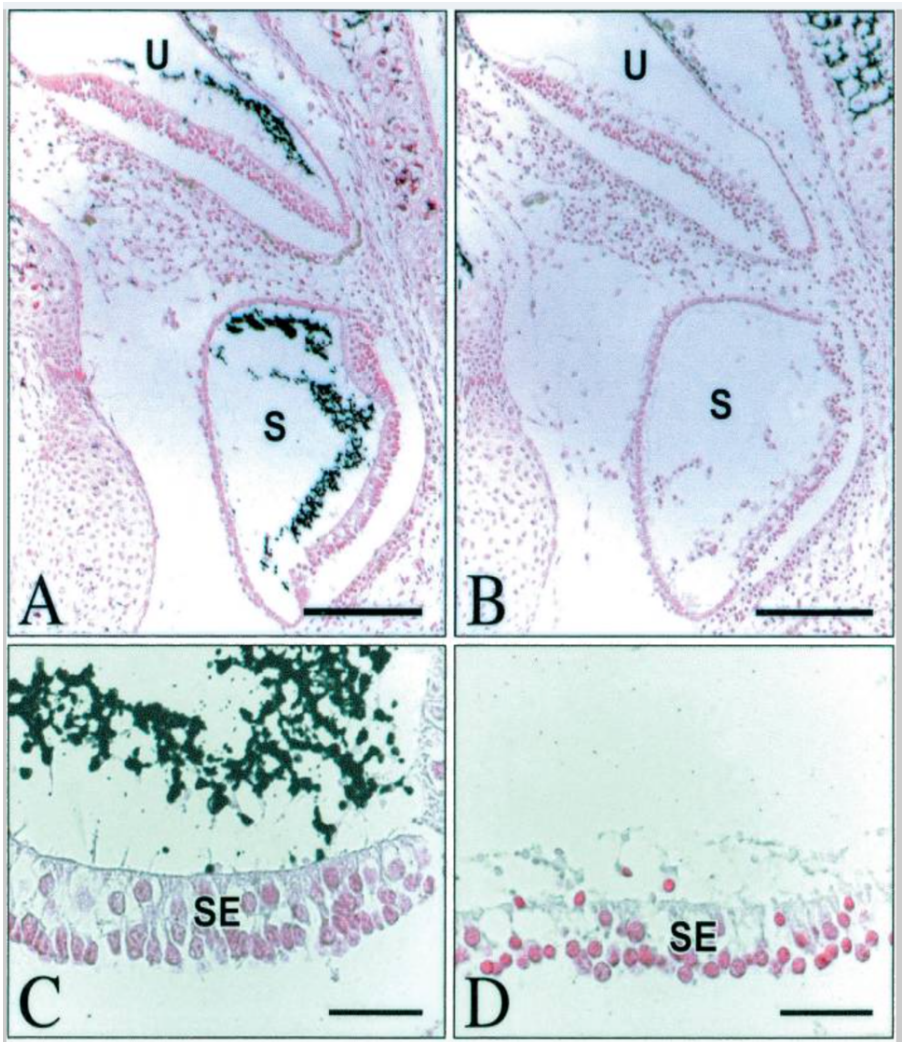
Nox enzyme	*Gene defects*	*Disease/ phenotype*
Nox2	Nox2* (gp91phox) p47phox*, p67phox* p22phox*, p40phox* Rac2*	Chronic granulomatous disease: susceptibility to bacterial & fungal infections; dysregulated inflammation; autoimmune disorders (arthritis, colitis); altered vascular phenotypes.
Nox1	Nox1*	Blunted hypertensive responses to angiotensin II; Altered colon epithelial homeostasis; VEOIBD.
Nox3	Nox3, Noxo1, p22phox	Otolith developmental defects causing deficits in balance & gravity perception.
Nox4	Nox4	Altered responses to cardiac stress or ischemia- reperfusion injury; Anti-fibrotic phenotypes.
Duox1	Duox1	Blunted allergic asthmatic phenotypes; Altered urinary bladder mechano-sensing.
Duox2	Duox2*, DuoxA2* DuoxA1/2 double KO	Congenital hypothyroidism; VEOIBD Susceptibility to Helicobacter infection.

*Mutations detected in man; Mouse mutant & KO models

Head-tilt phenotype in Nox3 mutant mice



Absence of otoconia in Nox3-/- mice

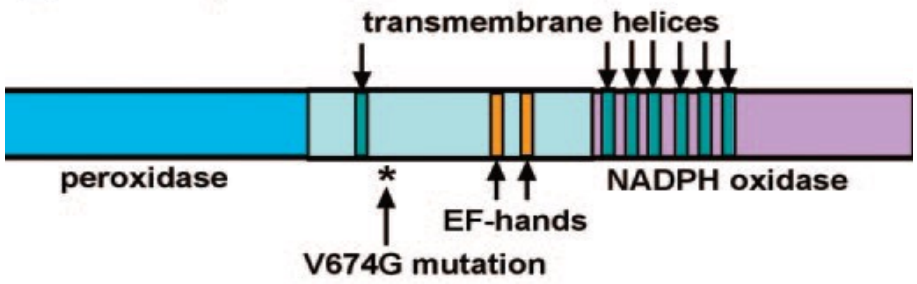


WT Nox3-/-

Paffenholz et al (2004) Genes & Development 18:486-91

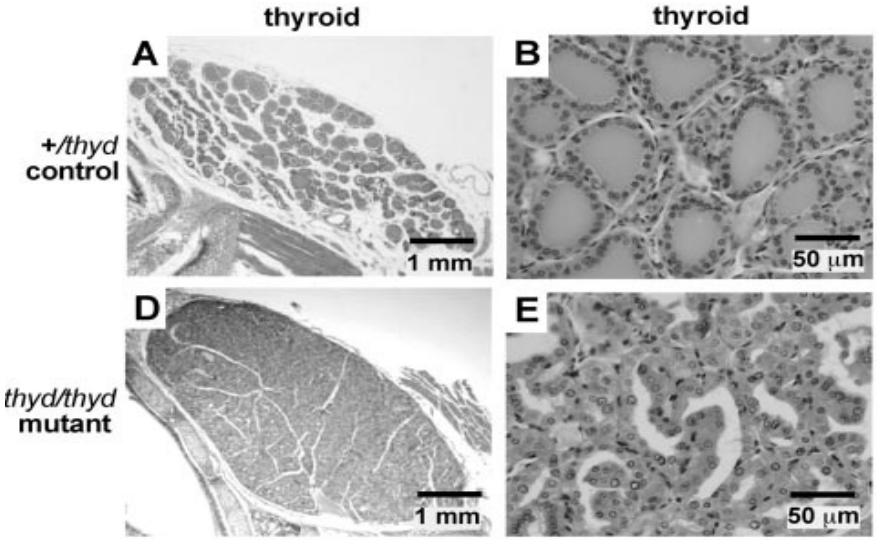
Duox2 mutant mice exhibit severe congenital hypothyroidism

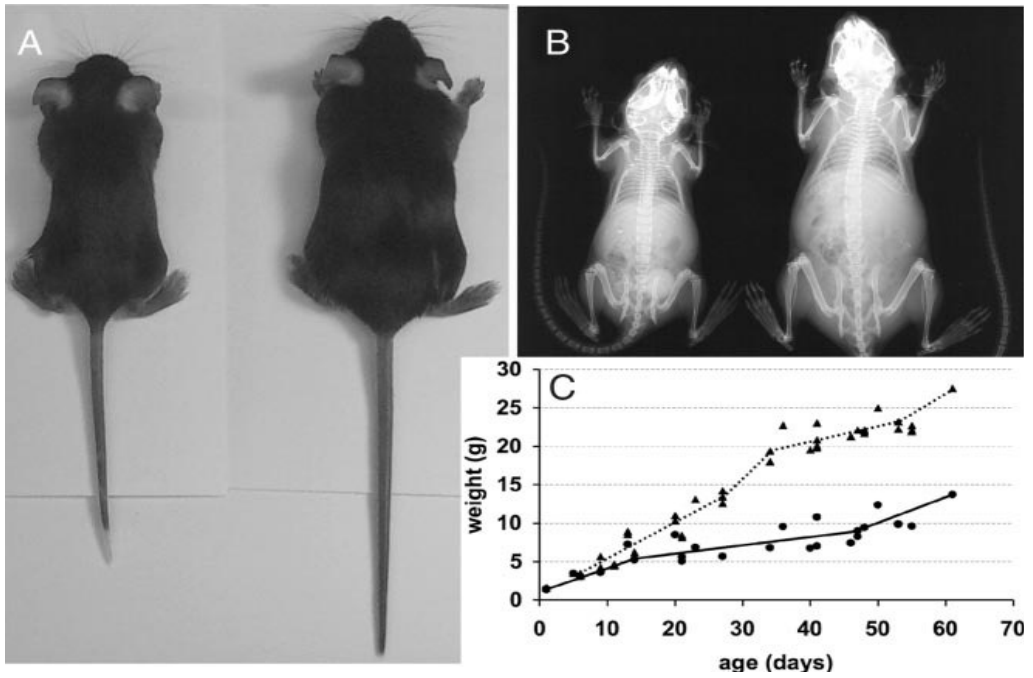
- 16 exon T → G
- Valine 674 Glycine:



Hypothyroidism:

- smaller size
- shorter bones
- reduced bone density
- hearing defects
- abnormal thyroid and pituitary gland
- TSH increased 100-1000x
- Reduced serum T4 and IGF-1





Johnson et al. Congenital hypothyroidism, dwarfism, and hearing impairment caused by a missense mutation in the mouse dual oxidase 2 gene Mol Endocrinol. 2007

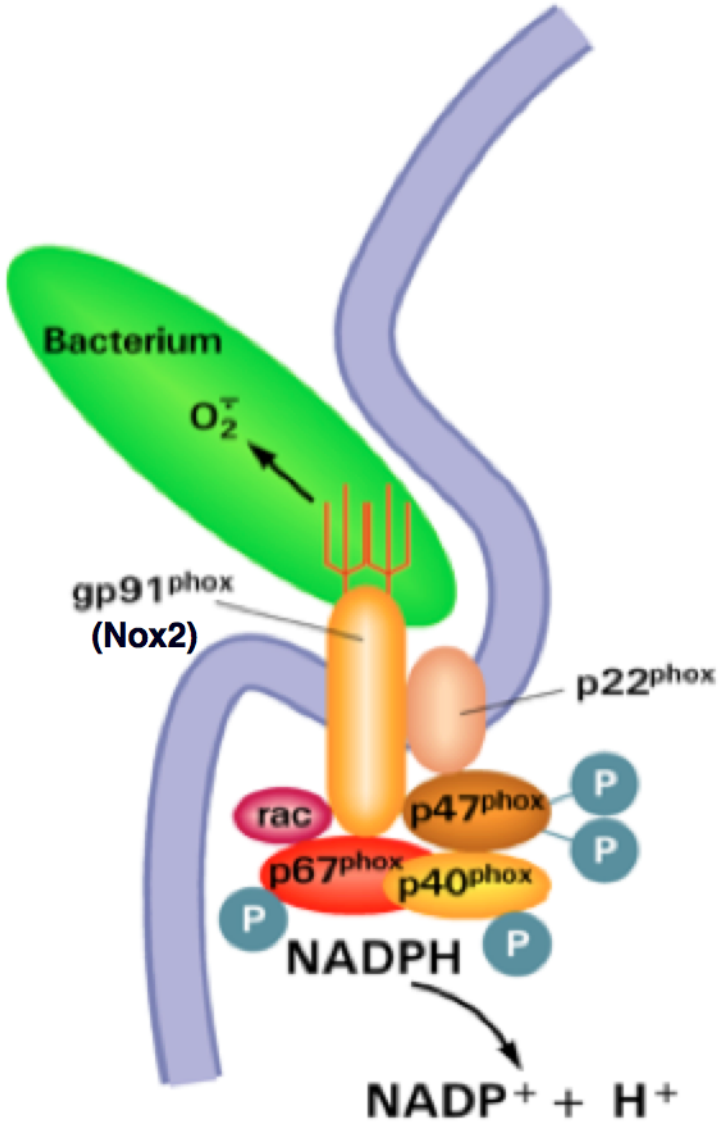
Roles of non-phagocytic NADPH oxidases in innate immune defenses-

- **Accumulate at high levels on epithelial surfaces (barrier function):**
  Nox1: colon; Duox1: respiratory, UG tracts; Duox2: GI tract, exocrine gland ducts.

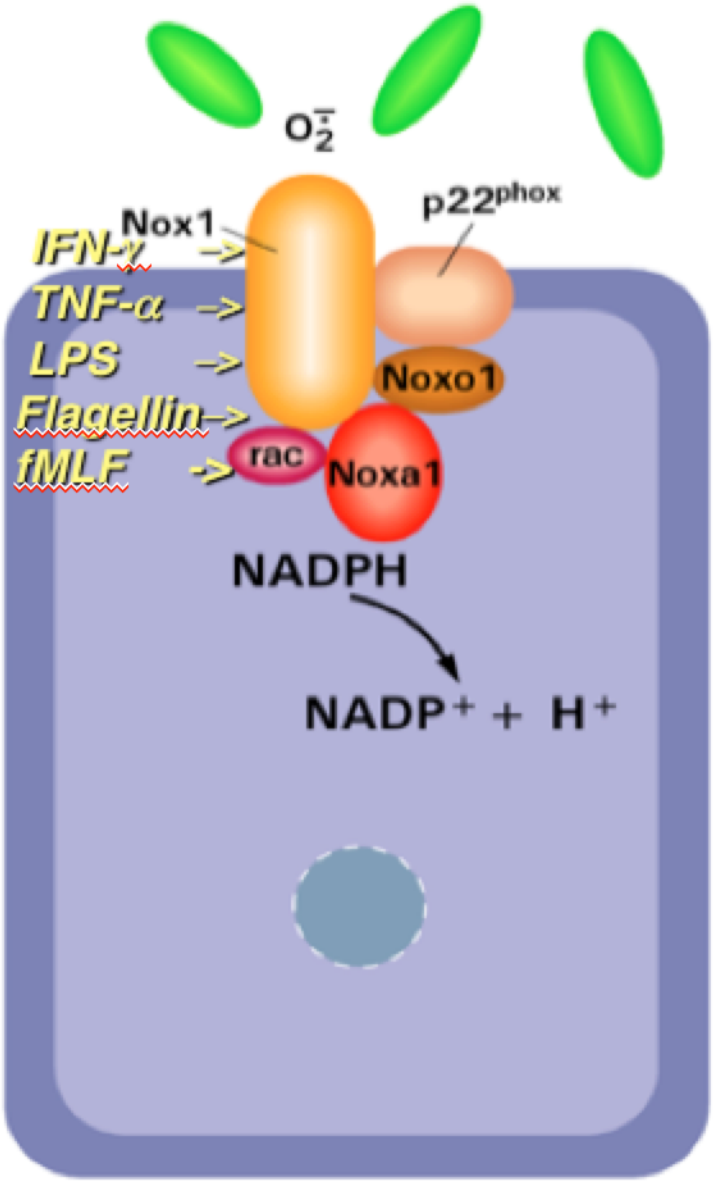
- **Responsive to microbial-, pathogen- or danger-associated molecular patterns (MAMPs, PAMPs & DAMPs):**
  Nox1: LPS, flagellin, fMLF; Nox4: LPS; Duox2: poly I:C, LPS, muramyl dipeptide, flagellin; Duox1&2: Histamine, ATP.

- **Induced by pro-inflammatory signals/ cytokines:**
  Nox1: IFN-γ, TNF-α; Nox4: TGF-β; Duox1: IL-4, IL-13; Duox2: IFN-γ.

The Phagocyte Oxidase

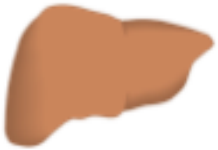


The Colon Oxidase



Leto and Geiszt, J. Biol. Chem (2004)

TGF-beta induction of Nox4 in pro-fibrotic and metastatic disease

Kidney		- TGF-beta mediated fibrosis - Hypertrophy in the diabetic kidney - Ischemia reperfusion injury
Lung		- TGF-beta induced proliferation and hypertrophy - MMP-1 expression and alveolar wall degradation (idiopathic pulmonary fibrosis)
Heart		- TGF-beta-depend cardiac differentiation - Angiogenesis - Cardiac stress (fibrosis from pressure overload)
Liver		- Nox4-induced oxidative stress in response to TGF-beta during chronic HCV infection (cirrhosis and hepatocellular carcinoma)
Breast		- TGF-beta induces Nox4-dependent migration and markers of the epithelial-to-mesenchymal transition (EMT)

The Epithelial-to-Mesenchymal Transition (EMT)

The EMT is a process in which polarized epithelial cells are reprogrammed to become dissociated and assume features of enhanced plasticity and motility.

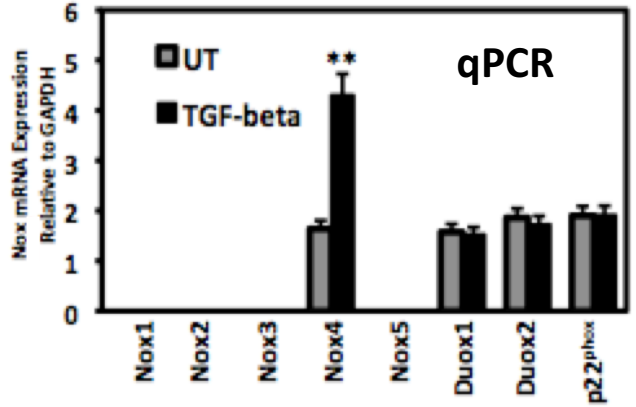
TGF-beta is considered a "master switch" for the induction of EMT

The EMT functions in a variety of physiological and pathological settings:

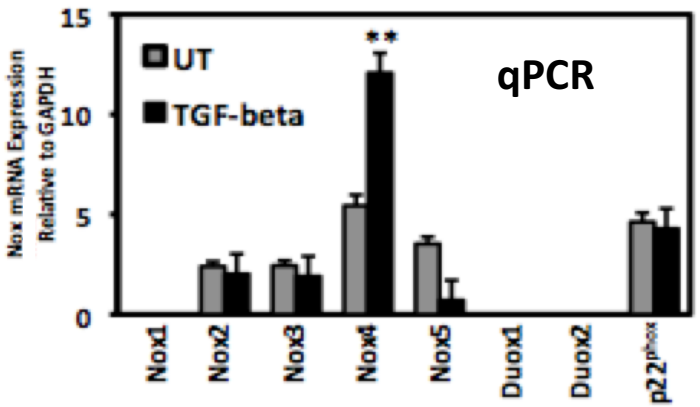
Physiological: development, differentiation, & wound healing
Pathological: fibrosis, carcinogenesis, & metastasis

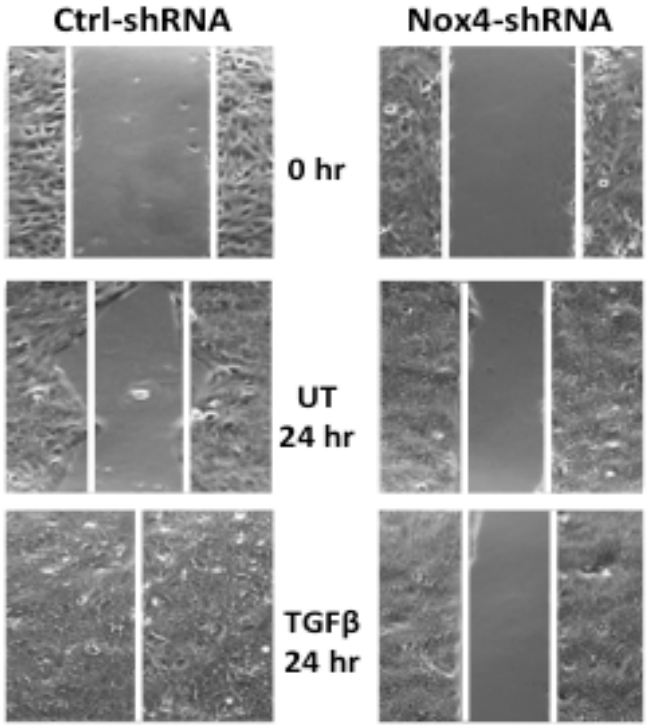
Nox4 promotes TGF-β-mediated cell migration and wound healing in normal and metastatic breast epithelial cells

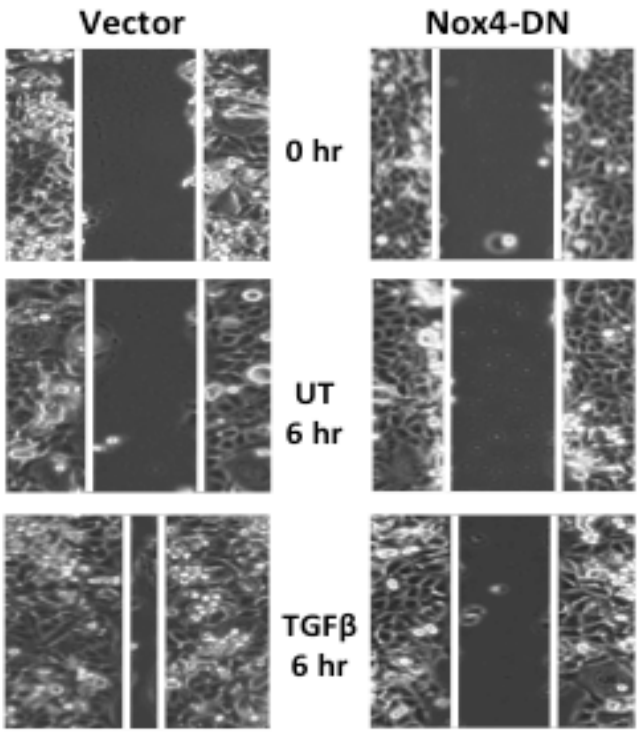
MCF10A
(Immortalized normal)



MDA-MB-231
(Highly metastatic)

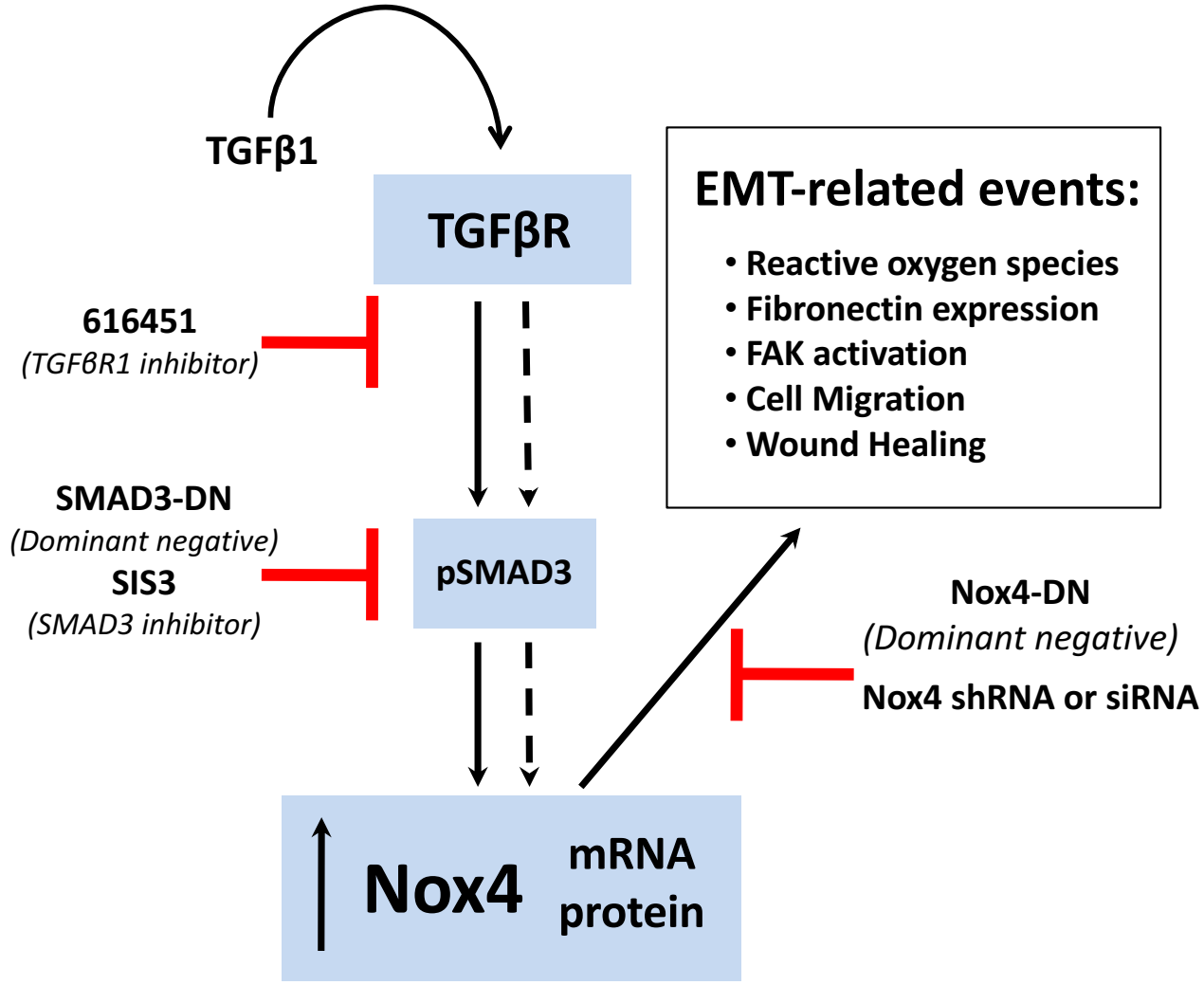






Boudreau, et al., (2012) Free Rad. Biol. Med.

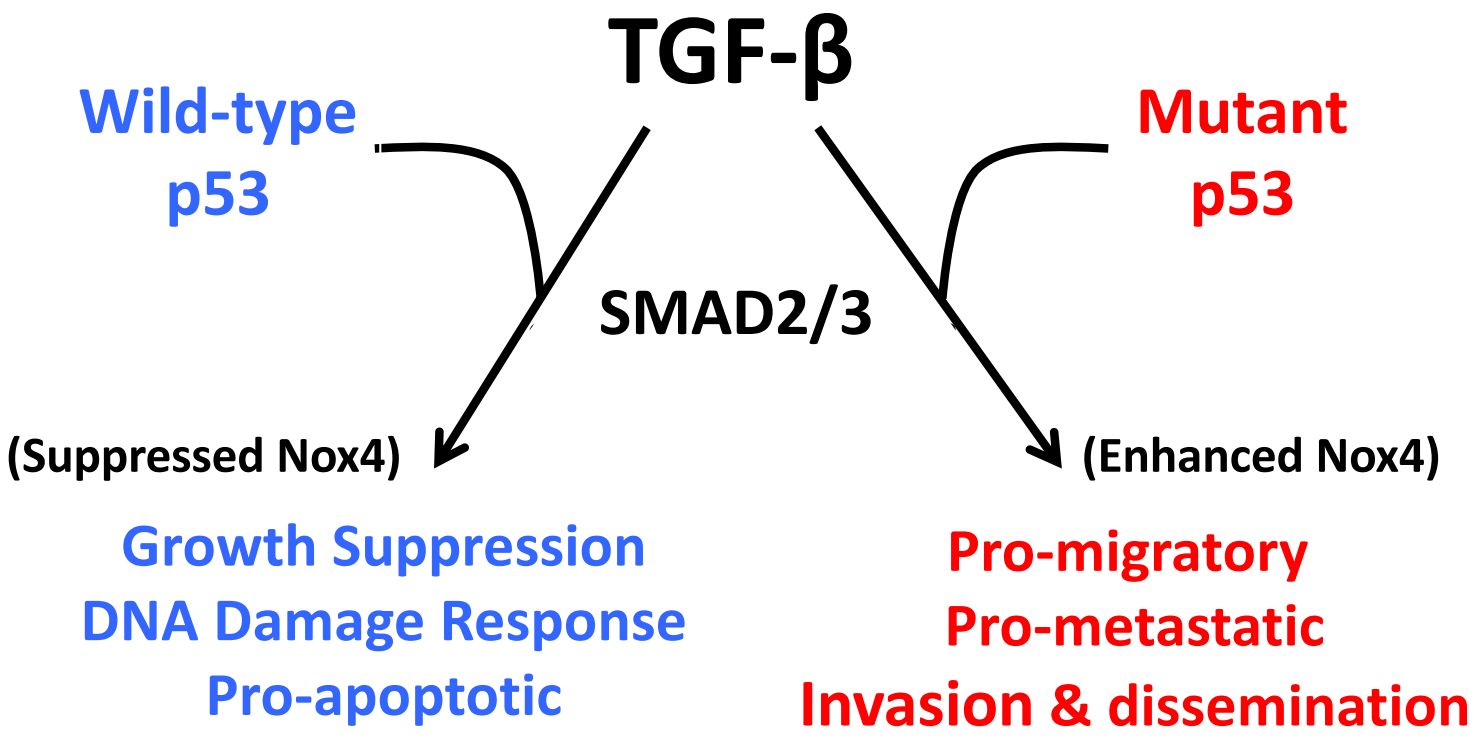
Role of Nox4 in the TGFβ/SMAD3-driven epithelial-to-mesenchymal transition (EMT)



What factors account for the enhanced migration of metastatic MDA-MB-231 cells?

Boudreau HE, *et al*. ***Free Rad. Biol. Med.*** *(*2012)

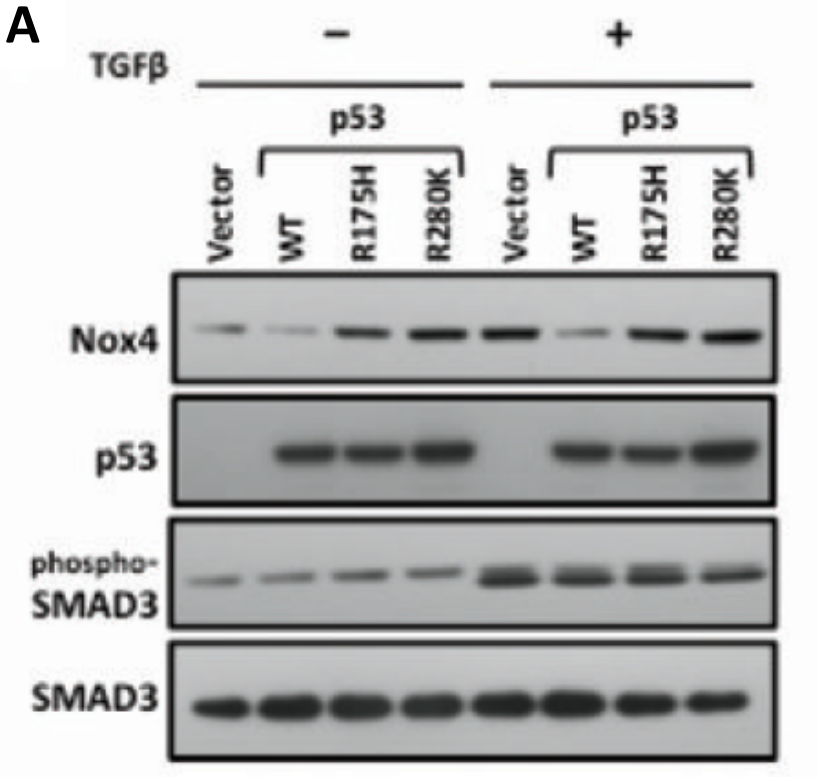
Convergence of p53 and TGFβ signaling: Divergent effects of wild-type and mutant p53

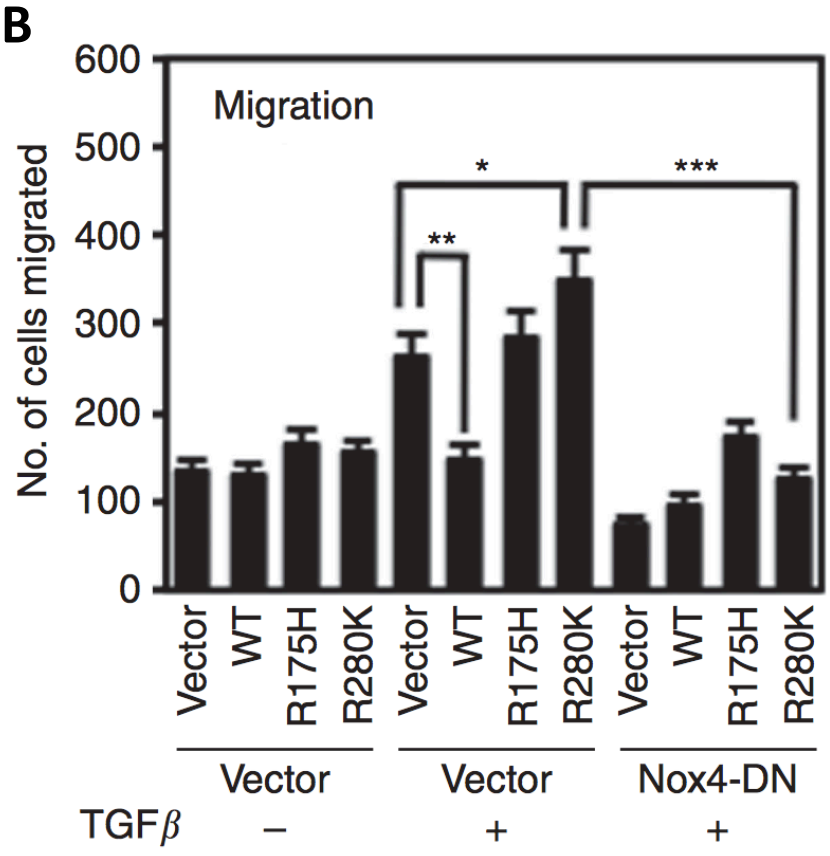


Adapted from: Adorno, et al., *Cell* 137, 87-98 (2009)

WT p53 suppresses Nox4 induction by TGF-beta, whereas mutant p53 supports Nox4 expression and cell migration

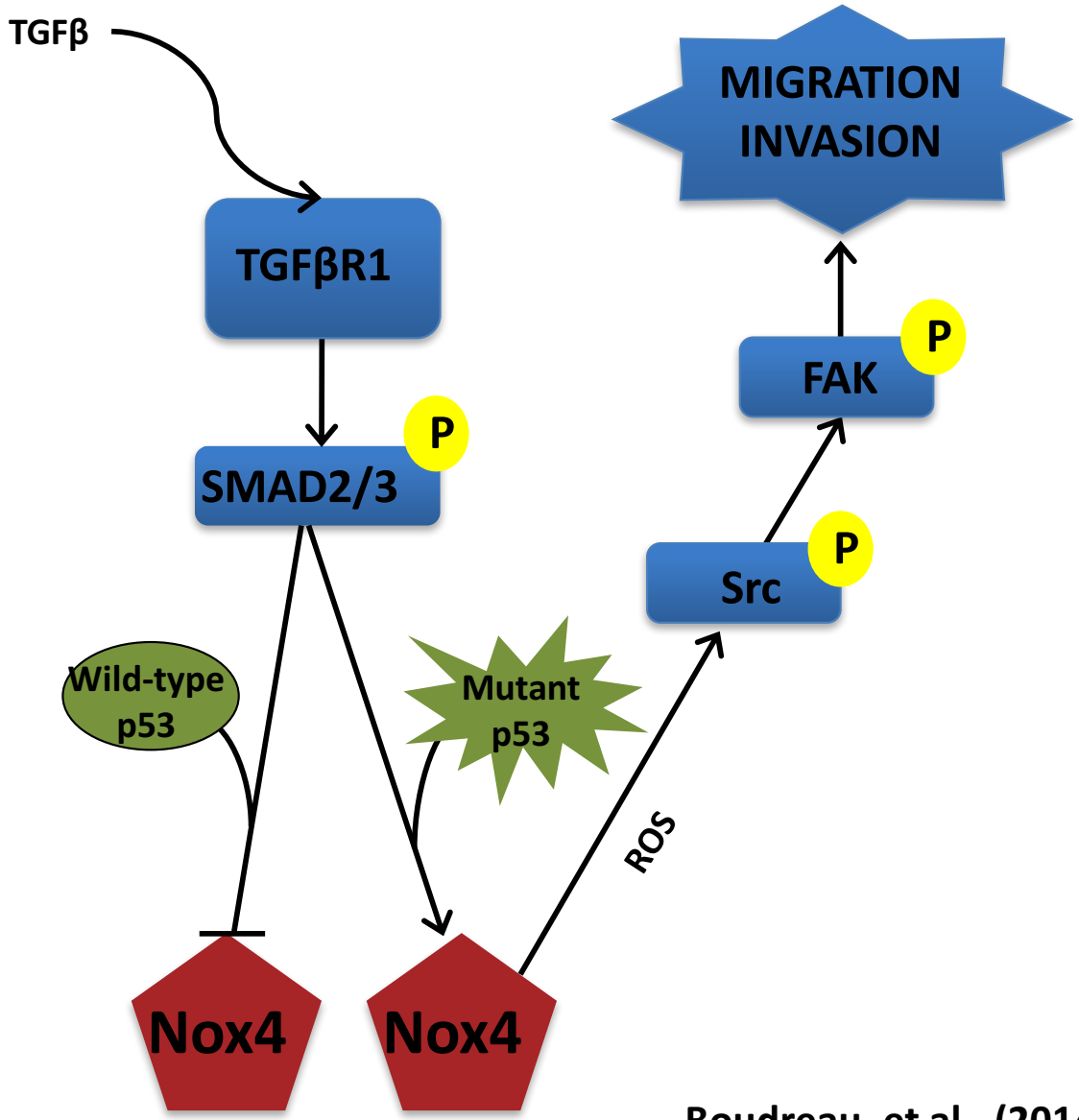
H1299 cells ***(p53-null lung epithelial tumor)***





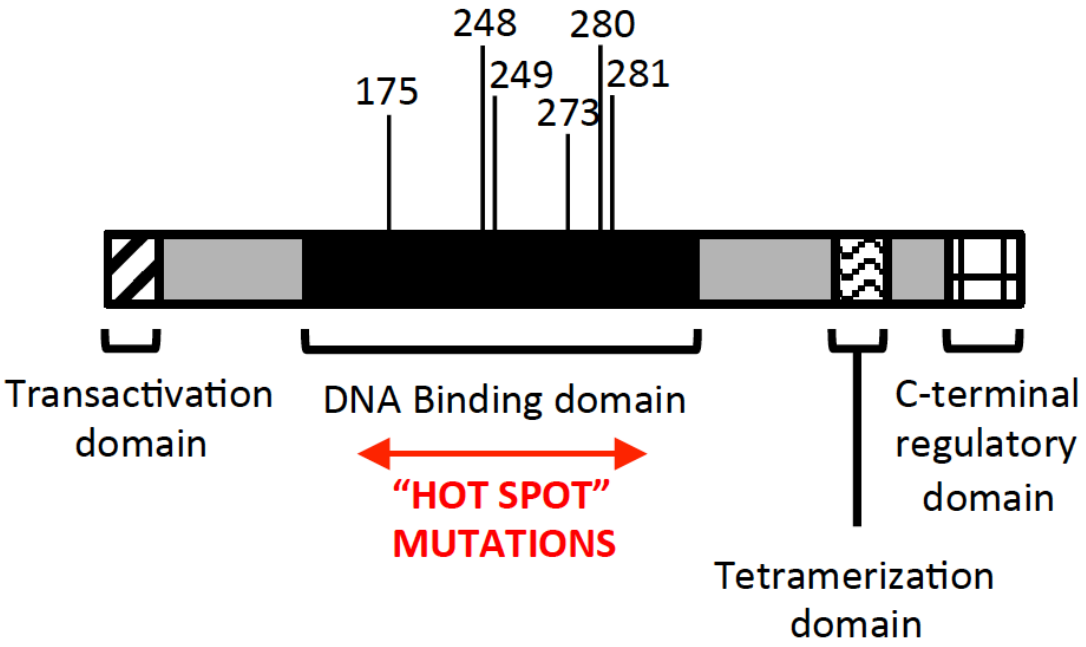
Boudreau, et al., (2014) Brit. J. of Cancer

Wild-type and mutant p53 have divergent effects on Nox4-dependent cell migration



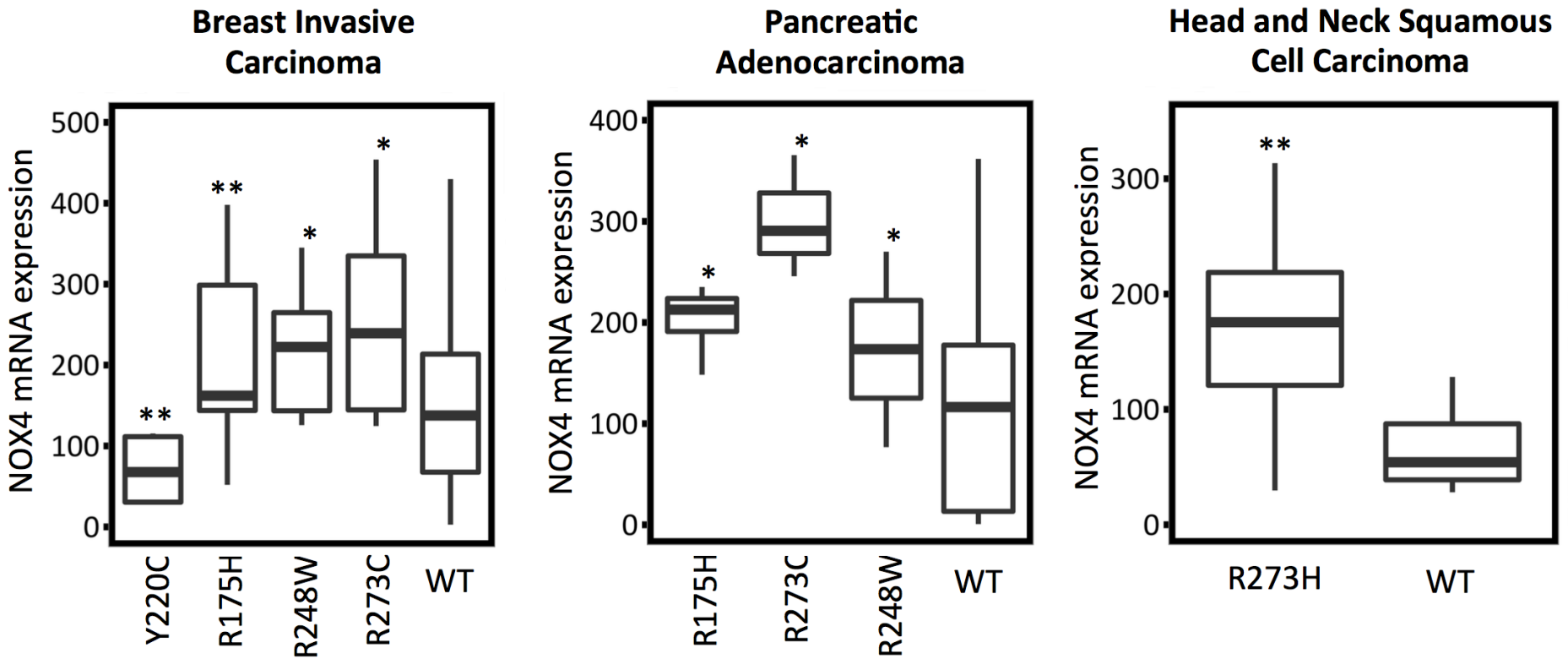
Boudreau, et al., (2014) Brit. J. of Cancer

p53 structure and common tumor-associated mutations



Tumor-associated p53 mutations reside primarily within the DNA binding domain which can produce a dominant negative effect or an acquired tumor promoting gain-of-function. All the above mutant proteins enhance Nox4 expression.

Nox4 expression correlates with p53 mutation status in primary tumors



TCGA database analysis by Wei-Feng Ma (unpublished)

Nox enzymes as potential therapeutic targets in inflammatory disease:

Fibrotic disease & tumor metastasis (TGF-beta-driven: Nox4).

Vascular disease (Nox1, Nox2, Nox4, Nox5).

Inflammatory bowel disease (Nox1, Nox2, Duox2).

Acute or chronic infection-related inflammatory disease (Nox1, Nox2, Nox4, Duox2).

Airway inflammatory disease, *i.e.*: asthma (Duox1, Nox2, Nox4).

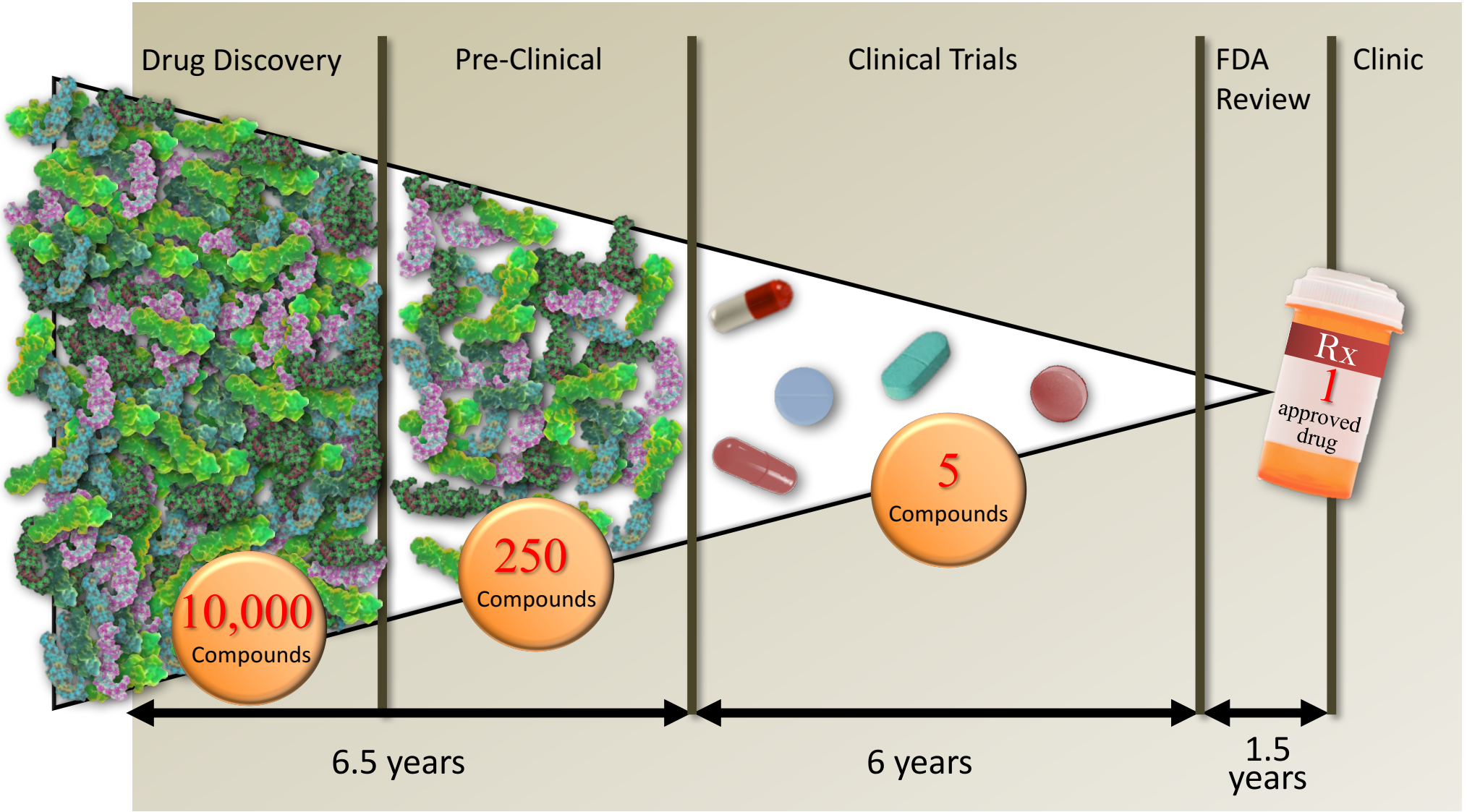
Acknowledgements

Howard E. Boudreau
Wei-Feng Ma
Agnieszka Korzeniowska
Jonathan J. Park

Journey to Discover NOX Inhibitors

Gal Wald, BA
National Institutes of Health (NIH)
National Institute of Allergy and
Infectious Disease (NIAID),
Laboratory of Host Defenses (LHD)

Nox2 Inhibitor Project: An Approach to Screening Compounds

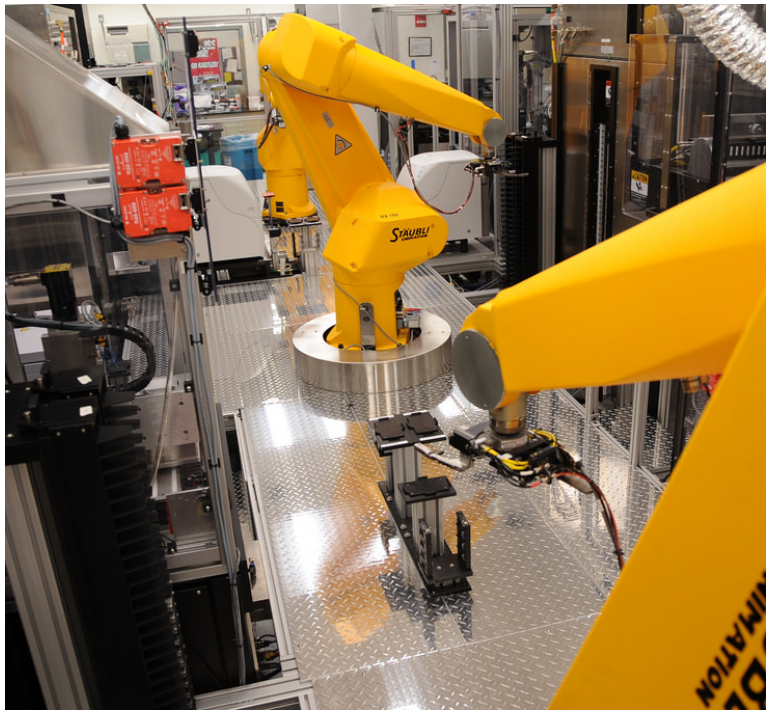


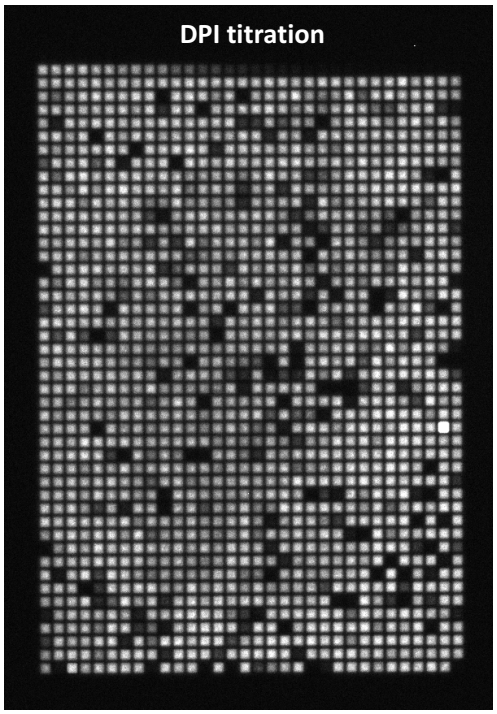
Primary Screen (done at NCATS)

Mark Henderson, Kyle Brimacombe, Ajit Jadhav, Anton Simeonov

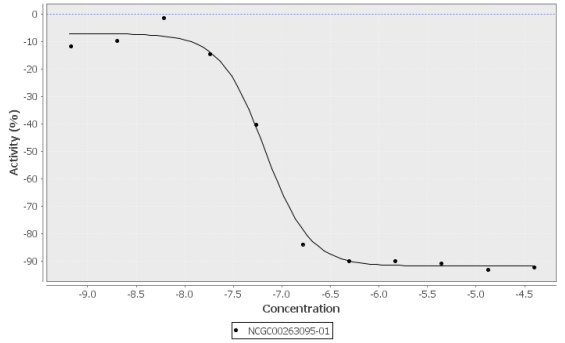
Luminol-enhanced chemiluminescence to detect reactive oxygen species following PMA stimulation of K562 cells stably expressing NOX2 and cytosolic factors

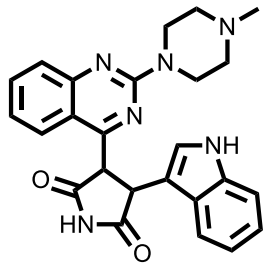
- 4426 compounds screened from the NCATS Pharmaceutical Collection (NPC) and Mechanism Interrogation Plate (MIPE) libraries
- Based on IC_{50} we received 102 compounds





1536 well plate

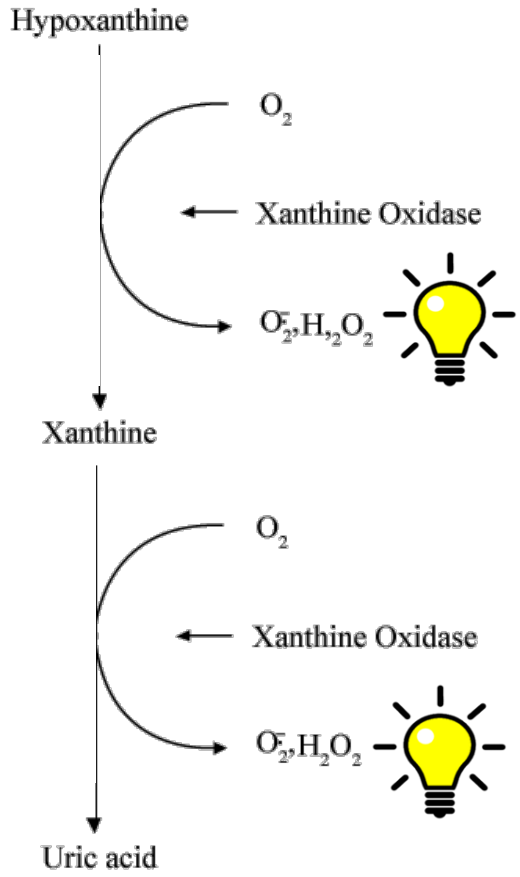


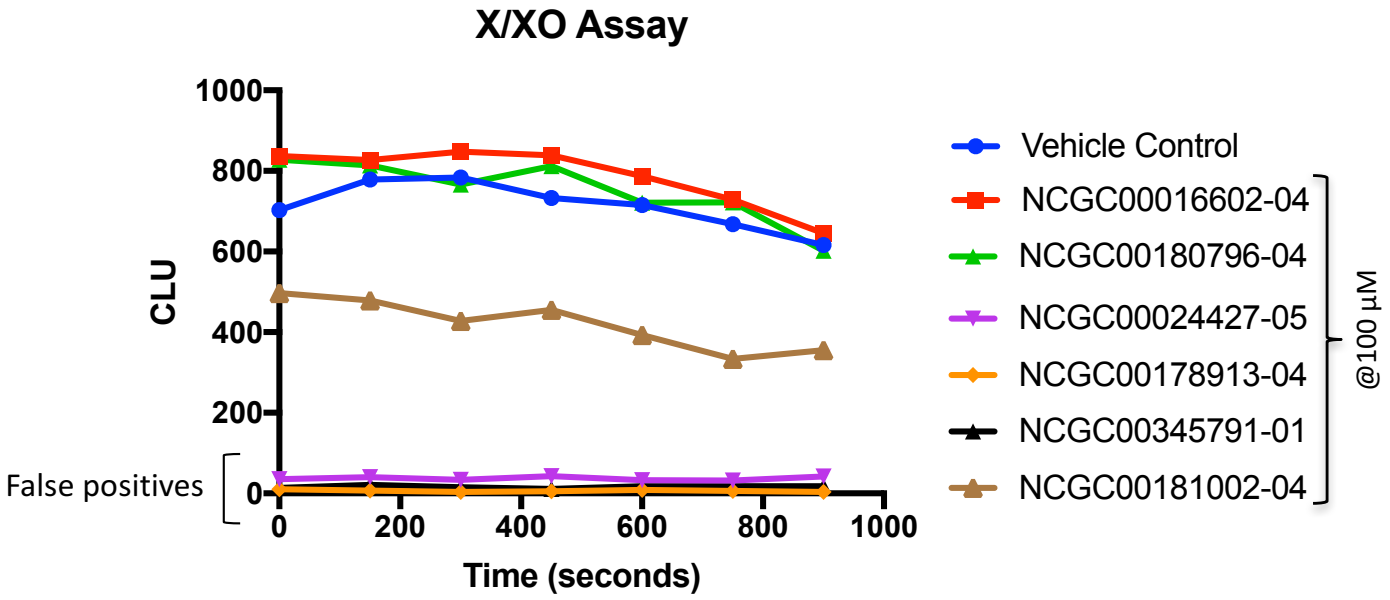


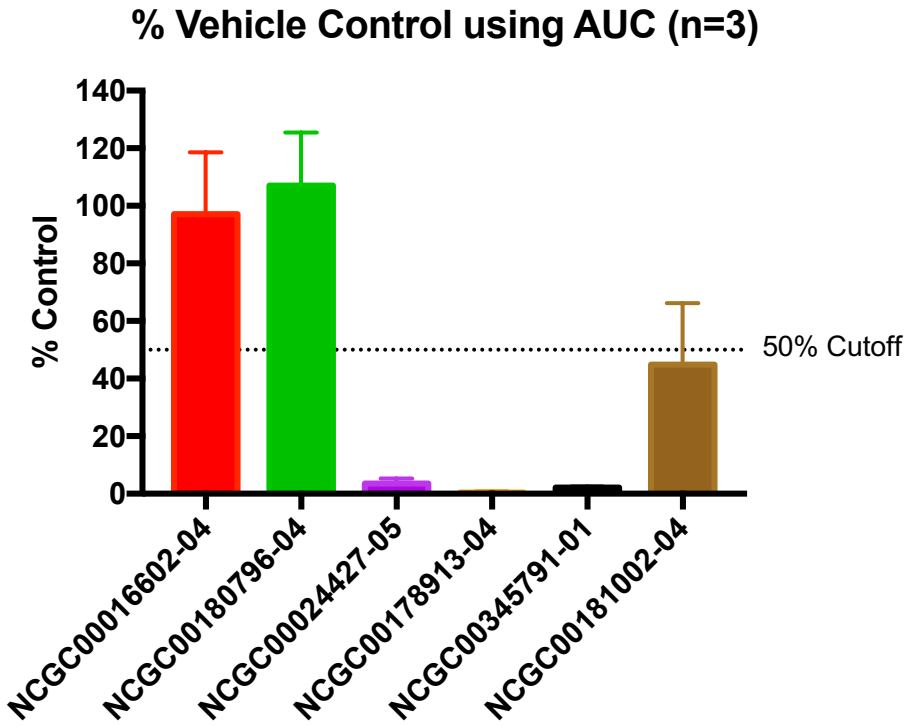
Sotrastaurin
(known PKC inhibitor)

Xanthine Oxidase Chemiluminescence Assay

- We used xanthine oxidase and its substrate hypoxanthine as a source of ROS to test for assay interference by NCATS compounds
- False positive screen for luminol-enhanced chemiluminescence assay used in primary screen. Screens for:
  1. Interference with signal detection
  2. Scavengers of ROS
  3. Non-specific inhibition of another oxidase system

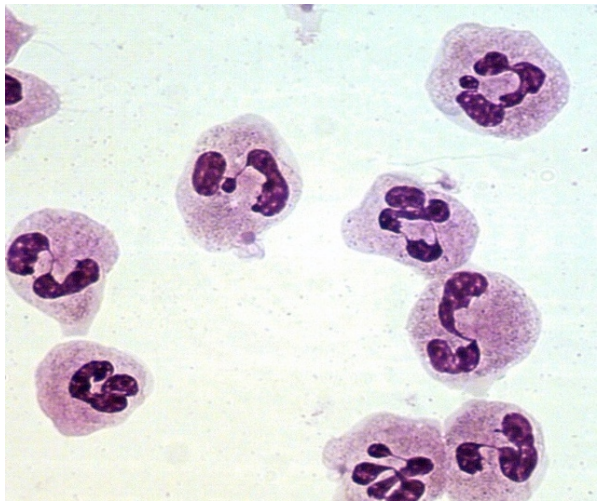






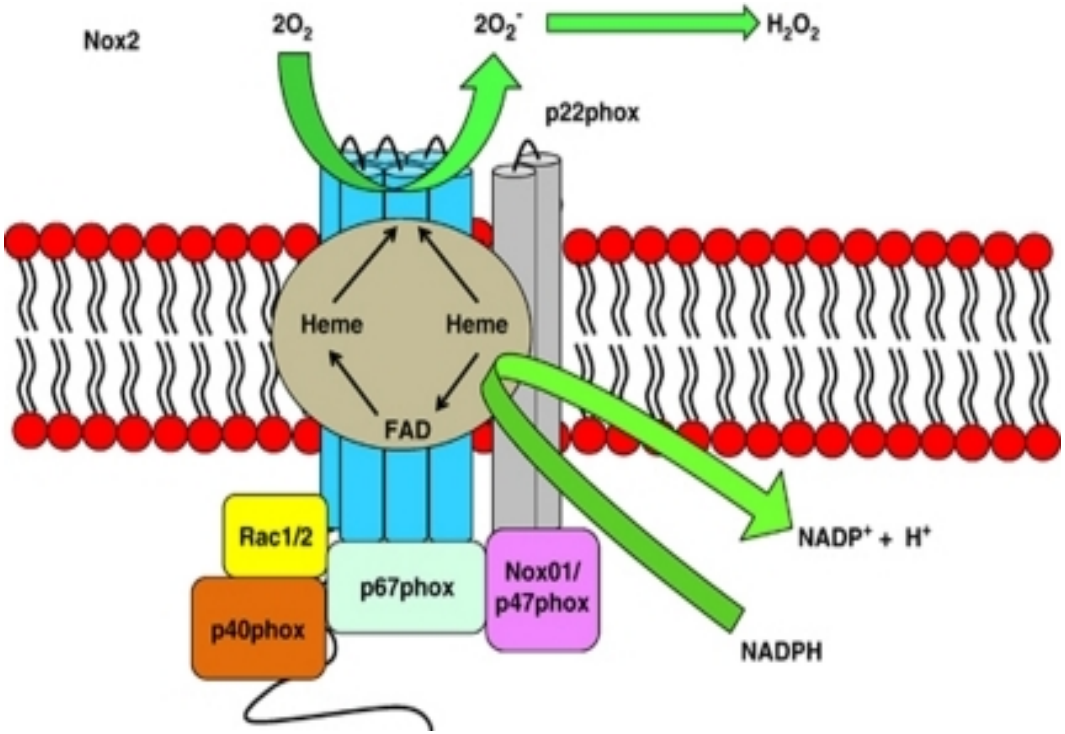
44/102 compounds had ≥50% Vehicle Control CLU

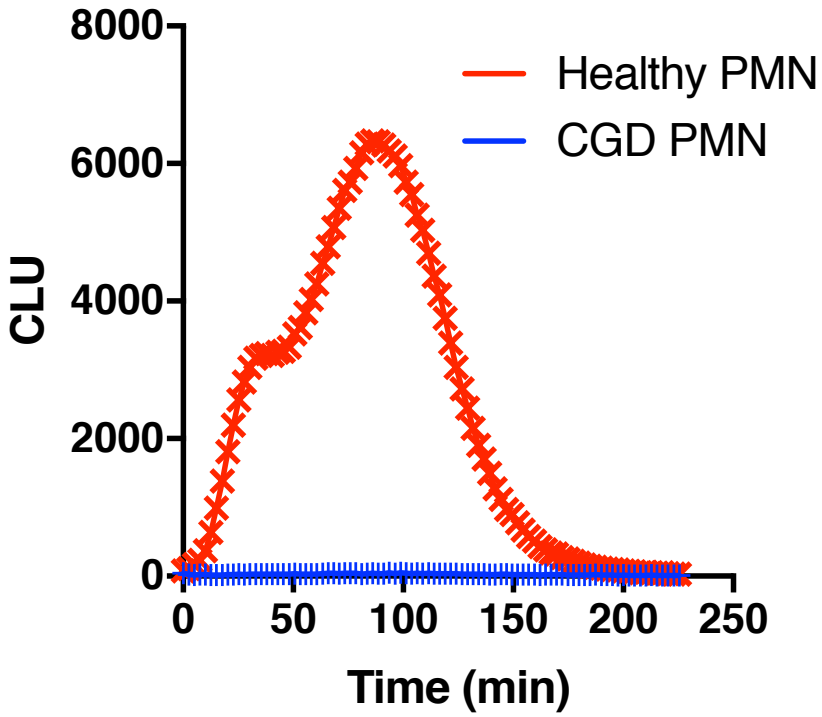
58 compounds either inhibited Xanthine oxidase, acted as antioxidants, or otherwise interfered with the assay



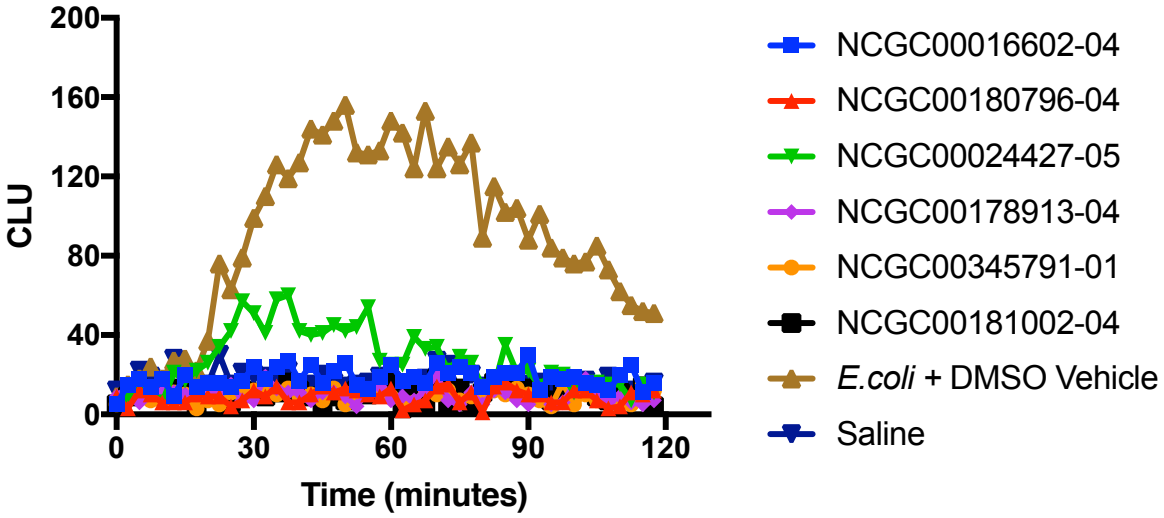
Human PMN Luminol-enhanced Chemiluminescence

Unlike the primary screen that evaluated inhibition in K562-Nox2 cells, here we evaluate the effect in live human neutrophils using *E.coli* as a stimulus

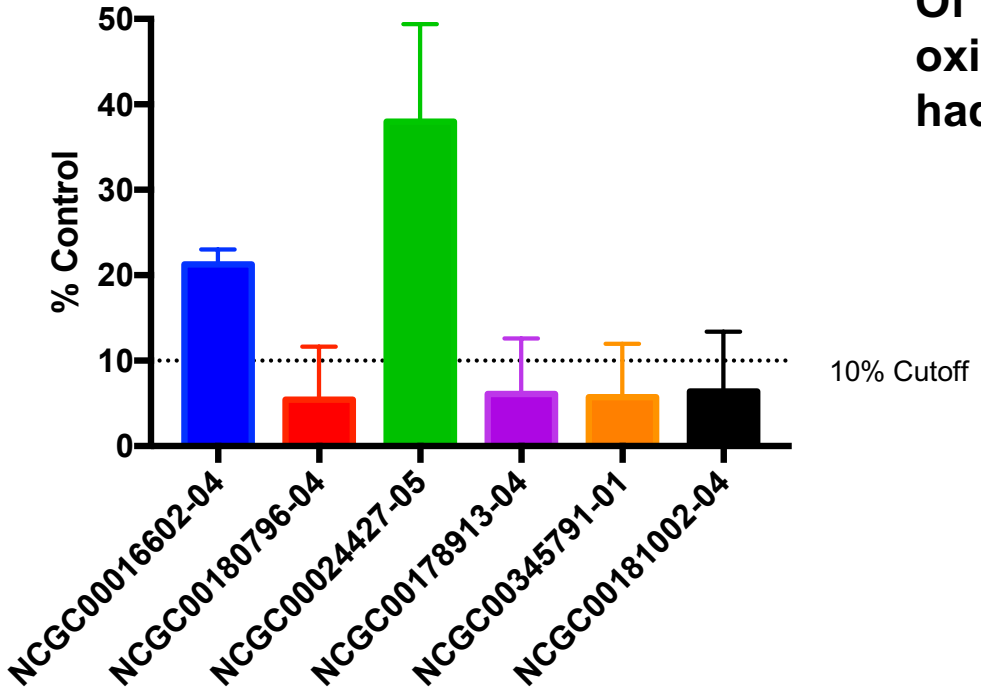




PMN Luminol-enhanced Chemiluminescence



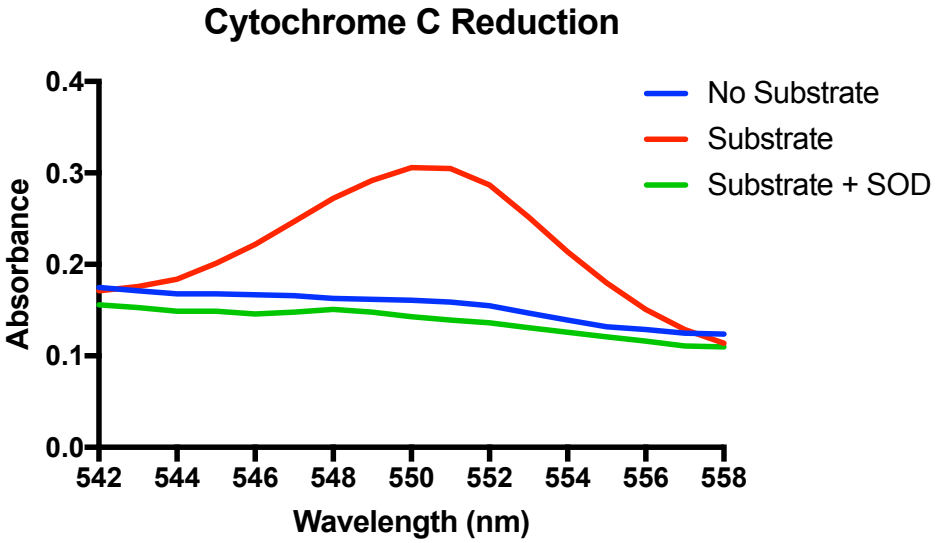
% Control using AUC (n=2)



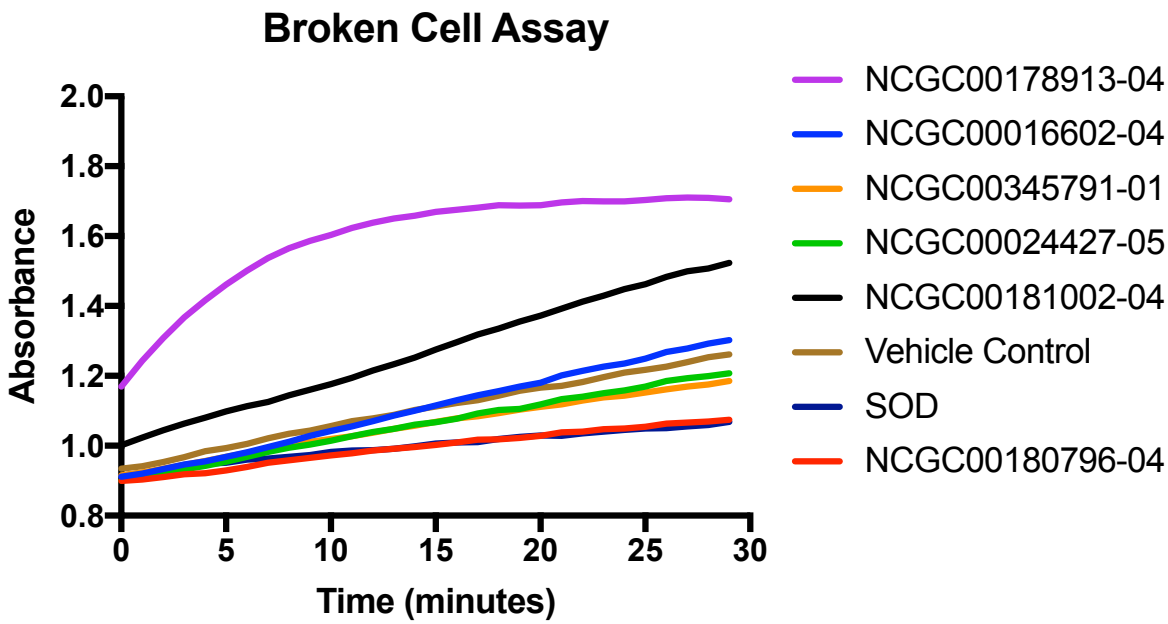
Of the 44 that passed the xanthine oxidase counter-screen, 36 compounds had ≥90% inhibition in human neutrophils

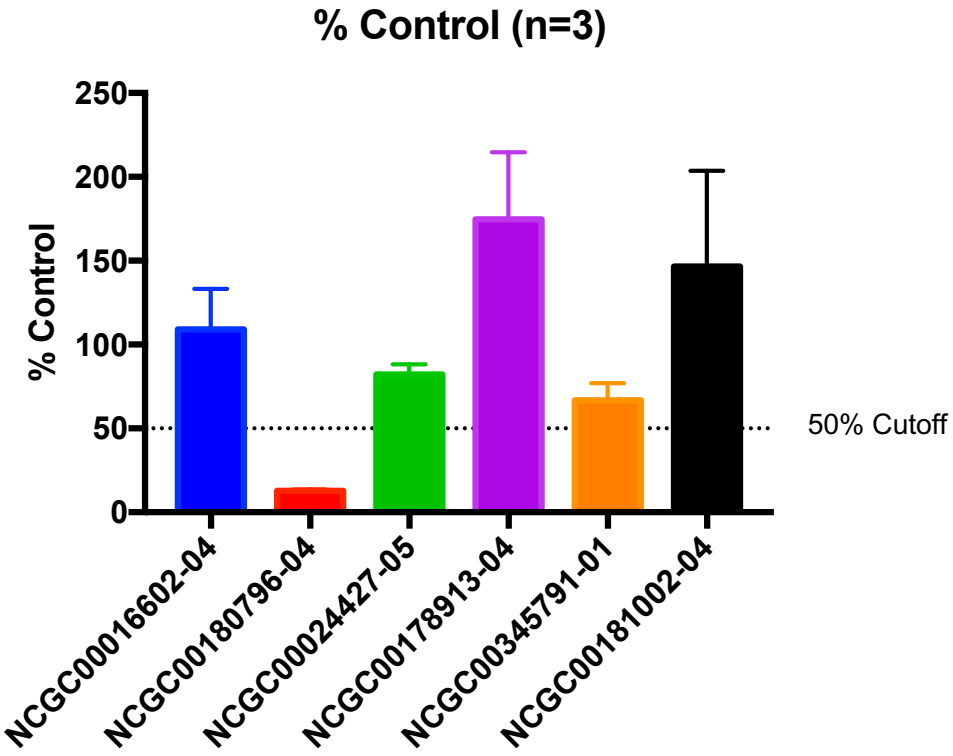
Broken-Cell NOX Assay

- Neutrophils were lysed and fractionated into a membrane component containing gp91^{phox} and p22^{phox} and a cytosolic component containing p47^{phox}, p67^{phox}, p40^{phox}, and RAC2
  - Addition of an amphiphile such as SDS directly activates the system
  - Bypasses membrane receptors stimulation and upstream signaling pathways
- Cytochrome c reduction to measure superoxide production



Reduced (no SOD) **oxidized (+ SOD)**





Of the 36 that passed both the xanthine oxidase counter-screen and whole cell human neutrophil assay, 10 compounds had ≥50% inhibition in the broken cell assay

10 Lead Compounds

At screening dose of 100 µM:

≤ 50% reduction in Xanthine Oxidase Assay

≥90% reduction in *E.coli*-induced neutrophil chemiluminescence

≥50% reduction in broken cell assay

Registration ID	Well ID	common name	K562-Nox2 IC50 (µM)	% Control XO	% Control PMN/E.Coli	% Control Broken Cell Assay	Broken Cell Assay IC50 (µM)	NOTES
NCGC00250408-01	D01	GSK-2126458; GSK 2126458;2126458/	1.5	55.2	0.7	40.7	~100	mTOR/PI3K inhibitor
NCGC00186465-04	C01	MK-2206; MK-2206;NSC-749607; MK-2	15.8	54.7	0.8	32.2	N/A*	Allosteric Akt Inhibitor
NCGC00180796-04	A02	Withaferin A	#N/A	107.0	5.5	12.9	10.1	steroidal lactone, antinflammatory, PKC inhibitor
NCGC00346635-01	F04	WYE-125132; WYE-125132	7.5	91.7	6.2	47.8	98.4	mTOR inhibitor, ATP-competitive, antiproliferative
NCGC00249346-02	E09	SB1518; SB1518; SB1518	16.8	78.8	6.7	41.6	18.6	pacritinib: JAK2/FTL3 inhibitor
NCGC00165865-02	F03	Penfluridol	7.9	87.4	7.6	49.3	N/A*	antipsychotic, diphenylbutylpiperidines
NCGC00345784-09	B08	PF-3758309	#N/A	64.0	5.8	45.1	61.7	PAK inhibitor
NCGC00347217-03	C10	#N/A	#N/A	53.1	5.6	45.7	N/A*	N/A
NCGC00346941-01	G07	ITK inhibitor; ITK inhibitor	16.8	50.9	6.2	46.7	66.5	Interleukin-2-inducible kinase inhibitor
NCGC00167507-04	H01	Lapatinib; ;Lapatinib; Lapatinib;GW-572	7.1	75.4	9.2	39.7	18.5	tyr-kinase inhibitor

***= apparent stimulatory activity at certain concentrations**

IC_{50} was then determined for each of these 10 leads in the broken cell assay

Conclusions and Future Directions

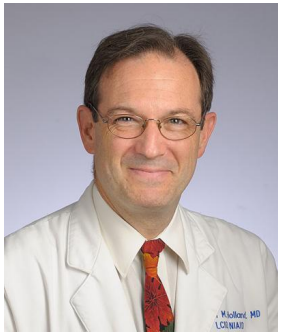
- Among 102 tested compounds, we found a relatively high false-positive rate due to antioxidant effects and inhibitory activity on cell activation pathways upstream of NOX2 (*e.g.* PKC).
- At this stage, we are gearing up with NCATS to screen the Molecular Libraries Small Molecule Repository (MLSMR) and other collections, totaling ≥500,000 compounds to be screened

Collaborators

NIAID

Kol Zarember, PhD
Tyra Estwick, RN
Harry Malech, MD
Steve Holland, MD
Nicole Rooths, RN

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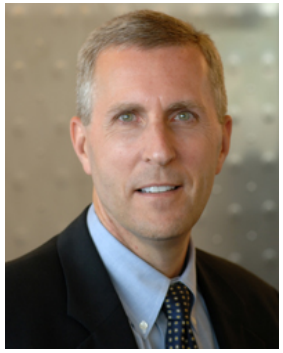


SAIC

Doug Kuhns, PhD